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ERRATA AND AUTHORS' EMENDATIONS

Page 9, line 23, "spikelet" should read "group."
Page 16, line 36, "*Triticum*" should read "*Agropyron.*"
Page 68, after line 22 insert "picture."
Page 84, last line, omit "Not seen."
Page 124, line 23, omit "15."
Page 140, legend of Plate 15, omit "Late rosette stage of Chinese colza seedling."
Pages 166, 168, 170, 175, 178, 180, and 181, Tables II, IV, VI, IX, XI, XIII, and XV, after column heading "ash" insert "expressed as percentages of dry matter."
Pages 183 and 184, Tables XVI, XVII, and XVIII, column 2, omit "per cent."
Page 193, line 16, "epidemic" should read "endemic."
Page 200, line 23, "0.005" should read "0.0005."
Page 236, footnote to Table XII, after "42 days" insert "and 60 days."
Page 246, line 23, after "apical portion" insert "of claval region."
Page 413, Table I, cloumn 10, footnote reference "e" should read "f."
Page 414, Table I, column 6, footnote reference "o" should be transposed to column 7.
Page 422, citation 4, omit "In press" and insert "no. 9, p. 235–243."
Page 452, Table II, line 1, footnote reference "a" should be inserted before all entries.
Page 479, line 32, "organism" should read "organisms."
Page 481, "26°" should read "25°."
Page 491, "Table XVII" should read "Table XVIII."
Page 508, Table I, column 3, lines 13 to 22, ".283" should read ".028."
Page 607, line 13, "molas" should read "molar."
Page 614, Plate 72, figure E, and Plate 74, figure E, "ae" should read "al."
Page 810, line 25, "Kostelezkya" should read "Kosteletzkya."
Page 815, line 32, "divini" should read "diveni."
Page 816, line 18, "hessitans" should read "haesitans."
Page 822, line 38, "Kosteleyzkya" should read "Kosteletzkya."
Page 828, line 32, "Kostelelzkya" should read "Kosteletzkya."

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FUSARIUM-BLIGHT (SCAB) OF WHEAT AND OTHER CEREALS[1]

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INTRODUCTION

The cereal crops—wheat, spelt, rye, and oats—and also some grasses are subject to attack by a number of fungi belonging to the genus Fusarium, of which the most common and most important is known, in its ascigerous form, as *Gibberella saubinetii* (Mont.) Sacc. The organism attacks each of the hosts named above in at least two different ways, producing two distinct pathological conditions. The first condition results from an attack on the root systems and the bases of the young and later of the grown plants, occasionally causing partial or entire wilting. The second condition results from an attack upon some of the parts above ground. This may be a rotting of the nodes, found on rye, wheat, and barley, or blighting of the heads of wheat, spelt, rye, barley, and, less commonly, of oats and certain grasses. In all cases the various attacks on the same host are independent of each other. A wheat plant may be attacked underground or on the head only or on both the roots and the head, and in some cases even on some of the nodes; but in all cases these infections are quite independent.

Up to the present time little attention has been given to these two forms of attack by Fusarium, and they have commonly been considered two different diseases caused by one or more unknown species of Fusarium.

However, the results of the work reported here prove conclusively that these two conditions are only two different phases of the same problem. This is in accord with views previously held by Selby and Manns (*11*),[2] Schaffnit (*8*), and Naumov (*5*).

This report, which is of a preliminary nature, deals primarily with the headblighting of wheat, spelt, rye, barley, and oats, as caused by *Gibberella saubinetii*, comparatively little attention being given in this paper to the rootrot caused by this organism. Nothing will be said

[1] In cooperation with the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 31–32.

here concerning other species of Fusarium connected with both phases of this problem or of their possible relation to the similar diseases of corn. While *G. saubinetii* is unquestionably the cause of headblighting of the cereal crops under most conditions and throughout the greater part of this country, it is equally true that under certain conditions and in some parts of the country other species of Fusarium are also responsible for the headblighting of cereal crops. The following organisms besides *G. saubinetii* have been isolated from blighted wheat, rye, oats, and barley heads or plants: *Fusarium avenaceum* (Fr.) Sacc., *F. herbarum* (Corda) Fries, *F. culmorum* (W. G. Smith) Sacc., *F. culmorum* (W. G. Smith) Sacc. var. *leteius* Sherb., *F. arcuosporum* Sherb., *F. scirpi* Lamb et Fautr., *F. solani* (Mart. pr. p.) Ap. et Wr., *F. arthrosporioides* Sherb., and *F. redolens* Wr. These species, while very seldom responsible for the headblighting of cereals, are not so unimportant in the rootrot problem of these crops. Indeed, some of them (*F. herbarum*, *F. avenaceum*, *F. culmorum*, and *F. culmorum* var. *leteius*) have, in my observations, proved to be as important as *G. saubinetii* in causing rootrot of the cereal crops.

There is extensive literature on this subject which can not be reviewed in this brief paper. Only a few of the more important citations are given.

THE DISEASE

COMMON NAME

In this country the headblighting of the cereal crops is generally known under the faulty name of "wheatscab." It is not a wheat disease alone, because it also occurs on spelt, rye, barley, oats, and certain grasses. And it is not "scab" because it causes no scabbing of the heads or of any part of the various hosts but rather blighting of the heads. The infected heads are perfectly normal and remain so except that they are blighted, take on the color of bleached straw, and later may be overgrown with the mycelium of the pathogen. Since the name "wheatscab" is faulty in a number of respects, the name "Fusarium-blight" is used in this paper.

GEOGRAPHIC DISTRIBUTION

The Fusarium-blight of cereals is more or less common throughout the central and eastern cereal-growing sections of the United States. It has been reported by the Plant Disease Survey for 1917, 1918, and 1919 from the following States: Maine, New Hampshire, Vermont, Massachusetts, Connecticut, New York, Pennsylvania, New Jersey, Delaware, Maryland, West Virginia, Virginia, North Carolina, South Carolina, Georgia, Alabama, Tennessee, Kentucky, Ohio, Indiana, Michigan, Illinois, Wisconsin, Minnesota, Iowa, Missouri, Oklahoma, North Da-

kota, South Dakota, Montana, and Oregon. It was looked for but was not found in the following States: Washington, California, Wyoming, Texas, Arkansas, Kansas, Louisiana, Mississippi, and Rhode Island. It has been reported from various parts of Canada.

In Europe the disease has been found in England, France, Italy, Germany, Austria, Holland, Denmark, Norway, Sweden, and Russia. In Russia the disease is common throughout the wheat- and rye-growing sections. In Asia it is very common in the Usurian provinces on the Siberian Pacific coast. It has also been reported from Australia.

ECONOMIC IMPORTANCE

The Fusarium-blight of the cereal crops injures the plants in several ways and is generally considered an important disease of these crops. It lowers germination of the seed and causes dying off or weakening of the young seedlings. Later it causes dying and wilting of fully grown plants, and finally it blights the heads, wholly or in part, thus preventing them from filling. The severity of the headblighting varies from a fraction of 1 per cent to 100 per cent, and the loss due to decrease in yield in individual fields and localities may vary from 0 to over 50 per cent.

The data concerning the economic importance of the disease are incomplete and inadequate. For some phases of the disease, and for most of the crops, they are entirely lacking. The meager information at hand on this subject is found in the Plant Disease Bulletin issued by the United States Department of Agriculture.[1] This covers only the losses caused by blighting of the heads of wheat and is given for only a few of those States where the disease is known to be present and common in one form or another. No information is available concerning the losses due to decrease in germination and the killing of seedlings and grown plants.

The total loss due to the blighting of the wheat heads by *Gibberella saubinetii* and various other species of Fusarium for the States reporting amounted to 10,620,000 bushels in 1917, according to the Plant Disease Survey. The States reporting highest losses were Ohio with 3,577,000 bushels, Indiana with 2,513,000 bushels, and Illinois with 2,288,000 bushels.

If the estimate of the Plant Disease Survey approximates the actual loss due to the blighting of the wheat heads in the States reporting, then the total annual loss for the United States is probably close to 20,000,000 bushels.

No definite information is available concerning the importance of the disease in Europe, especially in Russia, where it is known to be one of the most important and destructive of the cereal diseases.

[1] U. S. Department of Agriculture. Bureau of Plant Industry. Plant Disease Survey. Plant Disease Bulletin, Supplement 8, p. 21–27. May 1, 1920.

DESCRIPTION

In spite of the extensive literature on this subject, there is no detailed description of any of the various phases of the disease. In some discussions of the disease no symptoms are given; in others there is a brief description of only the last stages of infection, or rather, of the final results of infection. Because of this situation it seems necessary to describe the disease in detail, giving special attention to some symptoms which previously have been overlooked.

BLIGHTED SEED

Wheat kernels obtained from heads blighted or partly blighted by *Gibberella saubinetii* show marked evidence of the effect of the Fusarium attack and can be easily distinguished in a sample of grain, even when only a very small percentage of such kernels are present. Wheat seed from blighted heads exhibits one of three more or less distinct and definite pathological symptoms, depending upon the time of head infection.

(1) Kernels from heads infected early in their development, possibly during or shortly after the blossoming period, are small in size, being sometimes hardly two-thirds as long as the normal. They are pale greenish gray in color, badly shrunken, not firm, and very light in weight. As a rule, such kernels are never able to germinate. They may be heavily infected or even covered with the mycelium of the fungus if they developed near the point of infection, or they may be perfectly free from any fungus mycelium, if they have developed far above the point of infection where the food supply was cut off.

(2) Kernels from heads infected two or three weeks after the blossoming period may attain nearly a normal size, but they usually have a slightly shrunken appearance. They are grayish white or cream-white in color, soft and starchy in texture, and much lighter in weight than the normal kernel. In this case, also, they may be infested and even covered with mycelium, which is especially evident in the groove, or they may be entirely free from mycelium, depending on their position in the head with relation to the point of infection. The percentage of germination of kernels in this class is very low.

(3) The third class of kernels consists of those which have been infected shortly before or just after the head is ripe. Such kernels differ very slightly from the normal, except that they are partly discolored, pinkish spots being not uncommon on them. While it is true that *Gibberella saubinetii* is the most common cause of pinkish red coloring on kernels in all three of these classes, it must be remembered that other fungi, Macrosporium and Alternaria for instance, may in some cases cause this coloring of grain. Kernels of this last class usually germinate normally, but before the young plant has reached the surface of the soil, or before it attains any considerable size, it not uncommonly wilts and

dies as a result of infection from the kernel. In many cases, however, the seedling survives the attack and reaches full development.

Kernels of rye from blighted heads show symptoms similar to those described for wheat. The kernels which are directly attacked by the fungus in blighted barley heads become dirty brown in color and are lighter in weight than the normal kernels if the infection takes place at an early stage in development. Often barley kernels are found with salmon-colored spots on which there are masses of conidia of Fusarium. Oat kernels show much the same symptoms as barley, except that they remain lighter in color. In all these cereals, symptoms similar to those caused by Fusarium-blight may be caused by other agencies, such as the exposure of the grain to rain.

SEEDLING-BLIGHT

Seedlings from seed naturally or artificially infected with *Gibberella saubinetii* are subject to attacks by this organism at a very early stage of their development, and the visible symptoms of the infection may become evident at the time of the germination of the seed or only a few days later. The first symptoms appear on the young coleorhiza and coleoptile and consist of the browning and rotting of these parts. The coleorhiza and coleoptile, which always die shortly after the formation of the permanent roots and the appearance of the first foliage leaf, seem to offer a good medium for the establishment of the various species of Fusarium, which then penetrate into the tissues of the permanent roots and the first foliage leaf, causing the browning and rotting of the invaded portions. If the attack has proceeded successfully, the formation of the two lateral roots, in the case of wheat, is either prevented or these roots are destroyed before attaining any considerable size. The older or basal portions of the roots are sometimes pink in color, but they are usually brown to black. The lower portions of the roots continue normal and healthy until their food supply is cut off by rotting of the upper parts. Often the remnants of the kernel are heavily overgrown with the mycelium of the fungus, and in some cases they attain a dark carmine red color. The leaves above the infected portion, which seldom extends above the ground if the plant is still very small, become yellow and later brown, the discoloration beginning at the tips. If the leaves are over 6 cm. long they usually take on a light-green color and then collapse and wilt very rapidly, showing a blighted effect. In many cases the infection may be restricted to the primary roots, the coleorhiza and coleoptile, and even to the first foliage leaf. In such cases new roots are soon formed, the second and third leaves develop, and the plant may recover almost entirely from the attack, which is still restricted to the parts originally infected. Such plants, if examined three or four weeks later, will show no symptoms of the infection and will usually continue to develop normally.

FOOTROT

Careful examination of the underground portions of winter crops early in spring and of spring crops somewhat later in the season shows partial rotting of the roots, the bases, and, in some cases, the interior of the stems just above the bases. Various fungi may be found associated with this condition on the cereal crops, among which *Gibberella saubinetii* and species of Fusarium are common. No attempt has been made to obtain definite data on the relative frequency of occurrence of different species of Fusarium on root lesions and discolorations. This, of course, would be necessary before their relative importance as organisms inducing rootrot under field conditions can be determined.

The first evidence of the pathological condition of the roots of the cereals, whether the source of infection be the seed or the soil, is the same. The organisms first appear on the remnants of the kernel and follow some of the primary roots, causing rotting and browning as described above. When the crown and the crown roots are formed, the primary stem below the crown roots, now quite darkened and in some cases beginning to die, is invaded by the organism from the remnants of the kernel and the primary roots. Soon it, too, becomes brown and shows evidence of rotting. When the invasion reaches the crown it may stop, or, depending perhaps on the condition of the plant, it may continue, invading the central woody portion of the primary stem above the crown as well as the secondary stems and causing a browning of the woody portions. Rotting and browning of the scale leaves and of the sheath may also occur as a result of the invasion. How much of this rotting and discoloration of the underground portion of the cereal crops due to Fusarium species is parasitic and how much is saprophytic is not known. That some of these organisms are parasites is shown conclusively by the rotting of the roots next to the remnants of the kernel or next to the crown while their lower portions continue to be normal. It is shown also by the browning of the interior of the primary stem at and above the crown. The separation of discolorations and rotting of underground portions due to the parasitic and saprophytic action of the organisms concerned is unusually difficult, as large portions of the original underground parts of the plants eventually die even without any fungus invasion, and the presence of parasitic organisms may have nothing to do with it. Such is the case with the primary roots and the primary stem below the crown, and later with some of the crown roots themselves.

The amount of damage, if any, due to this invasion of the roots and other underground portions is even more difficult to determine. As a rule, the plants so attacked are at first small and stunted, but with the coming of sunny and warmer weather they usually recover and reach normal development, even when very badly injured. With the coming of favorable weather such plants may send out secondary roots or even

aerial roots, a development quite common in oats, and before long the effects of the attack may largely disappear.

ON STEMS OF GROWN PLANTS

Occasionally full-grown plants are killed by *Gibberella saubinetii* or by one of several Fusarium species just before or shortly after the time of blossoming. The fungus attacks the roots and the stem close to the ground, the first node usually being involved in the infected area. The part of the stem in contact with the ground and the roots below are rotted and are commonly pink or yellowish brown in color. This rotting of the base interferes with the water and food supply of the plant, and wilting of the entire plant is the result. Such plants become bent or broken over soon after they wilt and hence are easily recognizable in well-kept fields. When such plants are pulled up they break at the base, the roots always remaining in the soil (Pl. 2, A). It must be remembered, however, that wilting of the whole plant in very much the same way is caused by other fungi as well, for example by *Colletotrichum* sp., although in attacks by this fungus the base of the dead plant is a much darker brown or black in color.

This infection at the base of the plant may be due to any one of several causes. It may be only a continuation of the attack upon the young seedling or it may be the result of a new infection. Either the decline in vigor or unfavorable weather conditions may be responsible for the appearance of the disease at this time.

The succulent embryonic tissue just above the nodes of the various cereals is especially susceptible to attack by *Gibberella saubinetii*. Here the infection is usually restricted to the node or the area immediately next to the node, seldom, if ever, extending more than 2½ cm. in each direction. In such cases the portion above the infected node usually wilts and soon dies. Conidia may be formed under certain conditions on the node itself and on the infected part of the sheath coming out from it. This condition was first observed by McAlpine (*4, p. 305*) in 1896.

BLIGHTING OF HEADS

WHEAT.—The symptoms and effects of headblighting of different varieties of wheat are, in general, the same. The blighted head usually takes on the normal color characteristic of the ripe head of that variety or a slightly lighter color.

Blighting of the wheat heads can be detected with absolute certainty at a very early stage, three to four days after infection has taken place, provided that weather conditions have been so favorable as to enable the parasite to establish itself on the host and to begin its work of destruction.

The symptoms of blight infection as they appear on Marquis or some other of the beardless varieties are as follows: The very first sign of blight

infection is a slightly brown and water-soaked spot, 2 to 3 mm. in length, on the glumes. The veins appear more water-soaked and have a much darker olive-green appearance than the area between them. The points at which the infected glume or glumes are attached to the rachis soon show the water-soaked appearance also. The water-soaked area increases more or less rapidly, depending on weather conditions, until the whole spikelet is covered. It then spreads to the neighboring spikelets.

If the weather is dry the infection may remain restricted to one spikelet. At this time the glumes and the spikelets originally infected gradually begin to lose the water-soaked appearance, dry up, and take on the typical color of the ripe head of the particular variety. This drying up of the infected spikelets follows closely the advancing infection, which usually proceeds downward, as was first observed by Freeman (*2, p. 310*) in 1905. The healthy part of the head above the point of infection usually dries up and dies without passing through the water-soaked stage, because of the cutting off of the water and food supply by the fungus at the point of infection. In some cases, however, one or more vascular bundles of the rachis may remain free from the fungous invasion and continue to supply the uninfected portion of the head with water and food until the head has ripened normally and has formed fairly normal kernels. When infection proceeds down the stem, producing the same symptoms as on the head, it may sometimes reach as far as the upper node. Here, too, the whole or only one side of the stem may become affected, while the other side with one or more vascular bundles still normal may continue to provide moisture and food for the living portion of the head. Usually, however, especially in dry weather, the infection is restricted to the head; and most commonly only a part of the head is destroyed. This may be the upper, middle, or lower part, depending on the kind and point of infection. Infection of the rachis causes blighting or dying of the whole head above the point of infection. In such cases the dead spikelets shrink and become more closely appressed to the rachis, while the uninfected portions of the head continue their normal development to maturity and become robust, with spikelets well filled, thus making the difference between infected and uninfected parts still more striking.

The point of infection, even when the attack is in an advanced stage, can easily be located, especially if the weather has been favorable. It is usually covered at first with a short, cottony, slightly pinkish fungous growth, while the rest of the infected area remains free from such a growth. Later, if the weather is favorable, this growth extends farther over the infected area and becomes the substratum on which a layer of conidia develops. This layer of conidia may be smooth (pionnotes) or more or less granular (sporodochia), depending on the causal organism and the age of the infection. The older it is the smoother it becomes. The conidial masses, which were originally slightly pinkish, now become dark salmon to grenadine in color, depending on the causal

organism. The conidial masses tend to be more dense in the cases of infection by *Fusarium herbarum* and *F. avenaceum* and less so in the case of infection by *Gibberella saubinetii* and other Fusarium species. Because of the fact that at the bases of the spikelets moisture from rain or dew is held for a considerable length of time, the conidia are usually formed here, extending along the furrow formed at the line where the inner and outer glumes meet. In cases where the infection extends down to the upper node, conidia may be produced on the node also. They never form pionnotes but usually produce small sporodochia, which are generally abnormal in size and shape.

RYE.—The symptoms of headblighting of rye are very much like those of wheat, except that the water-soaked appearance is not so prominent. The infection seldom extends as far down as the second node before the plant naturally matures. Conidia are usually formed only at the bases of the spikelets and in the furrow formed where the inner and outer glumes meet and, to some extent, under the outer glumes. In moist weather, however, conidia may be formed throughout the infected area. Heads infected and killed at an early stage remain straight, while normal heads are slightly bent.

BARLEY.—The symptoms of blight on barley heads are usually different from those on wheat and rye, seldom resembling those on the latter. Usually only one kernel is killed, or occasionally several kernels in one row. In some cases the three kernels forming a spikelet are attacked and later, if conditions are favorable, the rest of the head is blighted. The first sign of infection is a small, water-soaked, somewhat brownish spot appearing at the base or the middle of the glume or on the rachis. The water soaking and browning spread in all directions from the point of infection, soon including the whole glume, the whole spikelet, or several spikelets, but the infection is by no means as uniform as it is in wheat and rye.

OATS.—The symptoms of headblighting of oats resemble those of wheat. Because of the structure of the panicle, however, the infection is usually restricted to one spikelet and is therefore not so conspicuous as it is in wheat or rye.

LIFE HISTORY OF THE CAUSAL ORGANISM IN RELATION TO PATHOGENESIS

The life history of the parasite, so far as it is connected with that of the hosts, has been followed by the writer through the entire year, and is here briefly outlined.

PRODUCTION OF SPORES

CONIDIA

Production of conidia upon the host plant is more or less common in all forms of Fusarium attacks on cereals. In many cases it may be so abundant that it leaves no doubt as to the real source of inoculum for subsequent infection in nature.

On seedlings.—When a wilted seedling is pulled out and portions of its partly decayed kernel or of the young stem are examined under the microscope, a great number of normally developed conidia can frequently be seen. In rare cases masses of conidia are also formed on the rotted stem above the ground. The number of conidia so formed will be still greater if any particles of organic matter like straw, old stems, or stubble happen to be near the wilted or heavily infected plant, since the conidia-forming growth will extend over them. This growth soon disappears, however, leaving no evidence of its existence.

On nodes and bases.—Formation of conidia on the infected nodes or bases of mature plants, while common, is never very abundant because of the rapid drying out of these parts.

On heads.—The formation of conidia on the heads of cereal crops, especially of wheat and rye, shortly after infection takes place is common and so abundant as to give them a very distinct pinkish or salmon color. In dry weather the formation of conidia is restricted to the area where the infection originally took place, this being usually the base of the spikelet where the rain drops collect and the moisture is held for a longer time than on any other part of the plant, except possibly in the sheaths. The spore formation under such conditions extends up the several furrows formed by the joining of inner and outer glumes and to some extent even between the glumes. In moist weather the conidia are formed in great abundance over the entire surface of the tissue through which the hyphae of the parasite extend. The latter send out conidiophores through the stomatal openings, forming at first small balls of conidiophores and conidia over each stoma. Soon these balls converge into a uniform layer (pionnotes) of conidia extending over a large portion of the head. The following observation in the field corroborates this fact.

Before June 29, 1918, the weather was dry and there were very few conidia formed on the infected rye heads in the University experimental plots. The last two days of the same month were rainy and comparatively cooler. Following this, conidia were formed in such abundance that all the infected spikelets were practically covered with a layer of conidia which gave them a distinctly pink or salmon color.

Dry, blighted rye, wheat, or barley heads without any conidia also produced conidia in abundance when placed on the ground under a screen and kept moist.

On dead organic matter.—Old straw and pieces of stems and cornstalks in fields where the year before the crop had been heavily infected with the disease were often found to show large pinkish areas bearing numerous conidia, some of which belonged to some of the species of Fusarium which were found parasitizing wheat and corn. This condition was especially common on cornstalks and wheat heads left in the field from the previous year and bearing the perithecia of *Gibberella*

saubinetii, thus confirming results obtained by Hoffer, Johnson, and Atanasoff (*3*) in 1918, when it was demonstrated that the hyphae present in the previously infected heads or cornstalks remain viable till spring, when they form new conidia and thus help the further propagation of the fungus.

ASCOPORES

Whenever the cause of the disease is one of the species having a perfect stage, as is the case with *Gibberella saubinetii*, the perithecia of this fungus are produced in great number on all infected parts, but especially on the pseudo-plectenchymatic structures, on which there has been more or less formation of conidia. Perithecia are formed on seedlings and infected kernels (observed only under greenhouse conditions), on the straw and the heads of the various cereal crops, and on the stalks, sheaths, and ears of corn. The ascospores play an important röle in the life of this organism, since they are likely to resist extreme weather conditions and furnish inoculum for the first infection in the spring.

DISSEMINATION OF SPORES

The experimental work on this subject is limited to a study of the agency of wind, and to some extent of rain, in distribution of conidia. Other factors may also play some röle in the dissemination of conidia and ascospores, but time did not permit a study of other factors.

BY WIND

In a rye field slightly infected with blight, numerous spore traps [1] were placed on stakes in vertical and horizontal positions, some on the ground and some at various heights, ranging from 3 to 8 feet above the ground, and exposed from 12 to 24 hours, then examined under the microscope. The number of *Gibberella saubinetii* conidia caught was very small when compared with the number of spores of other fungi, especially rust spores, that was found on each spore trap. *Gibberella saubinetii* conidia varied in number from none to eight on the traps set closest to the ground and especially on those placed vertically and facing the prevailing wind. Most of the conidia of *Gibberella saubinetii* were caught by the traps set on the ground. The statement that the conidia of species of Fusarium are wind-borne is not new. Saito (*7*), studying the atmospheric flora of Tokyo, found that Fusarium conidia are carried by the air in small number. The same fact has been reported by a number of other workers.

That the ascospores of *Gibberella saubinetii* are also wind-borne is shown by the following observations in the field. One of the rye fields under observation in 1918, consisting of several acres, was located on

[1] Common microscope slides were covered with a layer of glycerin, or glycerin with some vaseline, and were used as spore traps.

top of a hill. The field, which was only partly in rye, sloped at its west end rather sharply to the south and at the east end sloped gently to the south and east. The north side, the top of the hill, was fairly level and protected by a wind-break of trees. To the east and west also there were trees. The top or level part of the hill was sown with winter rye and the sloping parts with second-year alfalfa in which barley had been the nurse crop the preceding year. On the old barley stems left in the alfalfa field were a considerable number of *G. saubinetii* perithecia with viable spores. The only wind that could reach this field was from the south. The rye field was as uniform as could be expected in all respects except slope. The degree of headblight infection, however, was very different in the different parts of the field, although it was only a small and narrow strip of land. Blight was practically absent in the west part, which was surrounded on the north and west sides by wind-breaks. However, on the southwest edge there was considerable blight infection among the plants that were immediately next to the alfalfa field in which, as stated above, *G. saubinetii* was present and the slope was very steep. The east part of the field, which was protected on the north and east sides by wind-breaks, had, on the other hand, up to 5 per cent of blight, not only among the plants next to the alfalfa field but also throughout its south half, while its north half was free from blight. Knowing of no other factors that could account for this difference, the writer is inclined to think that the following is the possible explanation of the distribution of the disease. The west end of the field bordering on the alfalfa field where the slope was steep was infected only through the area next to this field, because the wind, lifting the spores from the alfalfa field, could not raise them into the upper air currents and so over the hill but deposited them against the slope before they could reach the rye plants on the level ground. Thus, only those rye plants were infected that were next to the alfalfa field. In the east part of the field the situation was different. The slope there was gradual and the spores needed to be lifted only several feet in order to be on a level with the rye field. Thus they could be easily carried to the rye plants even by the slightest air currents; and for this reason, perhaps, the infection in this part of the field was greater, although even here it was restricted to that half of the field which bordered on the alfalfa field. This indicated that the source of infectious material was the alfalfa field and that the infection extended only as far as the topographical conditions permitted the wind to carry the spores.

BY RAIN

The conidia produced at first are usually very loosely attached to the mycelial growth and are easily detached from it by wind, insects, and other agencies, while the conidia formed later and in pionnotes, as is commonly the case, stick together. However, if a drop of water is placed on the pionnotes the spores are set free with great rapidity and

force, as shown by the fact that they are driven around in the drop with considerable velocity. It is rather evident, therefore, that rain assists in the liberation of conidia from the pionnotes, and thus they are carried down to the ground or transmitted from plant to plant as the plants wave in the wind.

Insects, no doubt, may also play some rôle in the dissemination of Fusarium conidia, but time did not permit a study of their importance.

TIME OF NATURAL INFECTION

The first blight infection in nature takes place during the latter part of the blossoming period. It is, however, not the most severe one; the secondary infections following shortly after the first being the ones that are most destructive.

Several wheat, rye, barley, and oat fields, all located within 4 miles of Madison, Wis., were selected for experimental purposes during the spring and summer of 1918 and were examined every other day, beginning about one week before the period of blossoming of rye and two weeks before the blossoming of wheat, barley, and oats.

The following is a typical brief record of the observations on one of the wheat fields:

Station No. 2. Town of Burke, Wis.

Field of Marquis wheat on corn ground. Field in level open country. Soil sandy loam. Stand good.

June 22, 1918. Plants in blossom. No signs of blight infection. Throughout the field there are numerous cornstalks with a great number of *Gibberella saubinetii* perithecia with viable spores.

June 28, 1918. Wheat just passing blossoming stage. No signs of blight infection. Ascospores in masses are oozing from Gibberella perithecia.

July 7, 1918. First indication of blight infection apparent. It consists of a water-soaked spot on single spikelets, usually on single glumes.

July 15, 1918. All suspected first infections have developed into distinct blighting of the heads.

Following the first infection there may be as many successive infections as weather conditions permit.

This observation agrees with the results obtained with artificial inoculations. Inoculation of plants before blossoming and following the dough stage gave negative results. While the organism will attack and penetrate the heads and the kernels in them during the latter part of the dough stage and also after maturity, as demonstrated first by Schaffnit (*8*) and later by Naumov (*5*), if there is abundant moisture and warm weather, this can scarcely be spoken of as infection in the true sense of the word. Wheat plants which were just heading out, others which were just past blossoming, and a third lot which were in the late dough stage were inoculated under exactly the same conditions, on the same day, and with the same spore suspension. They gave the following results: The first and third lots remained healthy during the first

week, while the second lot showed 100 per cent severe infection and the third lot remained free from the disease until full maturity. Some of the plants in the first lot showed slight infection seven days from the time of inoculation, during the time when they were in blossom. These results show that the spores remain on the infected heads until the heads reach a susceptible stage before infection takes place.

SOURCE OF NATURAL INFECTION

An important source of infection is the seed used for sowing. Cereal seeds carry, externally, viable conidia of *Gibberella saubinetii*, as well as of *Fusarium* spp., and many of the kernels are internally infected with these fungi, as has been shown by Selby (*9*), Selby and Manns (*11*), Schaffnit (*8*), Bolley (*1*), Wollenweber (*12*), Naumov (*5*), and many others. Many times the writer isolated *G. saubinetii* and several Fusarium species from what seemed fairly normal wheat, barley, rye, and oat kernels, as well as from kernels from blighted heads of the same crops. In all cases *G. saubinetii* was the organism most commonly isolated. Seed so infected carries the organism to the soil, where it attacks the young seedlings if conditions are favorable. It passes the winter in the soil, preferably on the killed seedlings or other organic matter. In the spring it resumes its growth, producing new conidia which when carried to other parts of the plant cause head or node infection.

The perfect stage of this organism, which is formed in abundance on infected heads, straw, or cornstalks, is an important source of natural infection. The conidia of this organism, which are always produced in abundance on the infected heads and stems, are the chief, if not the only, source of secondary infection.

Whether *Gibberella saubinetii*, as well as the other Fusarium species attacking the cereal crops, is present in the soil at all times and for long periods of time, always ready to attack the susceptible hosts sown on such soils, is an important phase of this problem to which the writer has given no attention.

OVERWINTERING OF THE FUNGUS

The organism, because of its comparative resistance to cold and drying, overwinters in various ways. When introduced into the soil with the winter crops, it overwinters in the form of mycelium and conidia where these are formed on the killed seedlings and on other organic substances. It also overwinters in the form of mycelium in and on the seed, straw, heads, and cornstalks that have been infected with the fungus the summer before. The organism has been isolated from such plant parts kept out of doors throughout the winter and spring. During the winter

of 1918 it was frequently isolated from cornstalks fed to the cattle on the University farm and from cornstalks that had been taken out into the fields with the manure or for cattle feeding.

The mycelium of the organism present in infected straw and heads of wheat, rye, and barley when stored in the laboratory at room temperature and moisture was found viable after 12 months. In the infected seed it remains viable even after the second year.

The undeveloped perithecia of the organism, which are often found in the fall on the straw and heads of the cereal crops, on cornstalks and sheaths, and on many grasses, are another form in which this organism overwinters. In the spring these perithecia mature and form numerous ascospores, which are later liberated from the perithecia and carried to the various susceptible hosts. Mature ascospores in perithecia on wheat heads and cornstalks preserve their viability for over 8 months when kept in the laboratory at room temperature and moisture.

DESCRIPTION OF CAUSAL ORGANISM

TAXONOMY

The chief cause of headblight and one of the chief causes of rootrot of the cereal crops in the United States is *Gibberella saubinetii* (Mont.) Sacc. The following is a list of synonyms:

Gibberella saubinetii (D. and M.) S., 1879, *in* Michelia, v. 1, p. 513.

Gibbera saubinetii Mont., 1856, Syll. Gen. Spec. Crypt., p. 252.

Botryosphaeria saubinetii (Mont.) Niessl, 1872, *in* Verhandl. Naturf. Ver. Brünn, Bd. 10, p. 195, pl. 4, fig. 29.

Fusarium graminearum Schwabe, 1839, Fl. anhalt, v. 2, p. 285, pl. 6, fig. 7; Sacc. Syll. v. 22, p. 1483–1484, 1913.

Gibbera pulicaris (Fr.) f. *zeae maydis*, Rehm: Ascomyceten 381. From New Jersey, 8, 1875, J. B. Ellis.

Fusarium roseum Autorum.

Fusarium tropicalis Rehm, 1898, *in* Hedwigia, Bd. 37, p. 194. Is probably a synonym of *Gibberella saubinetii* according to Wollenweber (*12*).

Gibberella tritici P. Henn., 1902, *in* Hedwigia, Bd. 41, p. 301.

Fusarium rostratum App. and Wollenw., 1910, *in* Arb. K. Biol. Anst. Land u. Forstw., Bd. 8, p. 30.

MORPHOLOGY

PERITHECIAL STAGE.—The following description of the perfect stage of this organism, given by Wollenweber (*12*), is adequate:

Diagnosis.—Perithecial stage: Perithecia scattered or gregarious, ovoid to subconical, free on the surface of the host as well as embedded in mycelium, or on a tubercular plectenchymatic stroma, which may either push in sphaerostilbe-like bodies through

the surface of the host or remain endophytic, 150 to 250 by 100 to 250 μ. Peridium smooth and small-celled at the basal part, but large-celled, verrucose occasionally, with protuberance-like projections of cell groups near the apical end, black to the unaided eye (turning red-brown with acid reaction), dark blue with transmitted light except the almost colorless often rather prominent beak; asci up to over a hundred in each perithecium, intermixed with a few celled paraphyses; ascospores, 8 in one row or irregularly in two rows, subdorsiventral, fusiform, slightly curved, tapering at the ends, ochreous in masses; largely 3-septate, 20 to 30 by 3.75 to 4.25 μ (up to 5 μ in diameter in germination, indicated by constrictions at the septa).

CONIDIAL STAGE.—In shape the conidia (fig. 1) strongly resemble the conidia of *Fusarium culmorum*. but they lack the constriction toward the base so prominent in *F. culmorum*. They differ also in being longer and more slender and in having thinner walls and less prominent septa; conidia typically, sometimes up to 100 per cent, 5-septate, 45 to 65 μ by 4.2 to 5.5 μ; 3-septate, 35 to 45 μ by 5 to 5.5 μ; seldom 4-septate; rarely 6-, 7-, or more septate, 60 to 75 μ by 4 to 5 μ; ochreous in mass. Chlamy dospores absent. Carmine red pigment on starchy, neutral media.

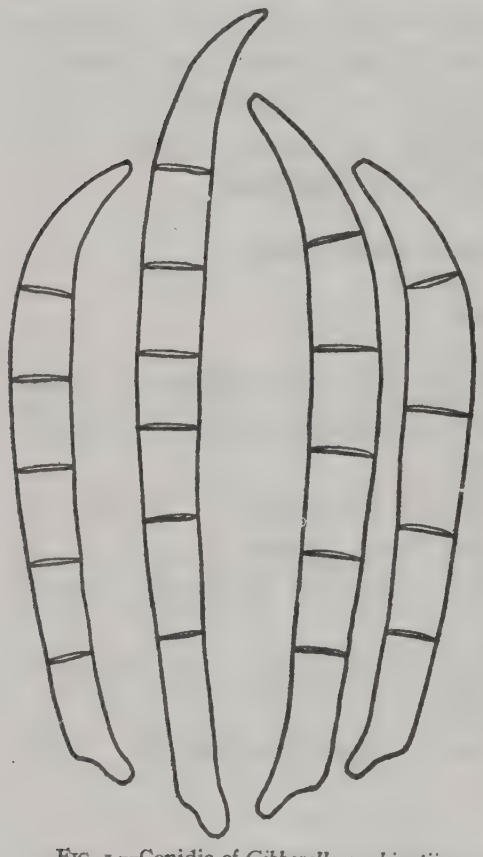


FIG. 1.—Conidia of *Gibberella saubinetii*.

HABITAT.—This species is one of the most widely distributed species of Fusarium within the temperate zone, causing headblight and rootrot of wheat, emmer, rye, oats, spelt, and corn in the United States, Germany, Russia, Italy, Denmark, Sweden, and probably elsewhere. Wollenweber isolated it from berries of *Solanum tuberosum* near Berlin, Germany. C. A. Ludwig isolated the same from *Ipomoea batatas* in storage at La Fayette, Ind. The writer found the perithecia of the fungus on Bromus, timothy stems, clover, and alfalfa, and also on *Triticum repens* which had been plowed under. The fungus was also isolated from asparagus stems collected at Baraboo, Wis., by Mr. E. H. Toole. According to Saccardo (*6, p. 513*), the fungus occurs on dead stems of Angelica, Asparagus, Beta, Clematis, Conium, Cannabis, Convolvulus, Cucurbita, Gyneria, Phytolacca, Scirpus, and Stipa, and on branches of Buxus, Coronilla, Fraxinus, Gleditschia, Juglans, Robinia, Rubus, Rosa, and Ulmus in Europe, Algeria, North America, and Australia. A. D. Selby (*10*) adds Emmer, Trifolium, and Medicago as new hosts. It has been found also on *Glyceria aquatica* in Germany, on rice in Japan and Italy, and on *Triticum spelta* in S. Paulo, Brazil.

METHOD OF PERFECT STAGE DEVELOPMENT

IN NATURE.—A limited study of the field conditions under which the perfect stages of some Fusarium species which parasitize the cereal crops and numerous grasses are formed showed that those conditions are as follows:

(1) Successful parasitism of the fungus on some host. The perithecia are formed usually and preferably on those dead parts of the host which have been parasitized.

(2) Successful conidia production. Conidia production on the infected substratum, root, stems, or heads always precedes the formation of perithecia, since the latter are formed more readily on the crust or plectenchymatic layer formed by the conidia-bearing hyphae and the germinated masses of conidia themselves.

(3) Presence of moisture. No perithecia will ever be formed in the absence of sufficient moisture, and their formation will be delayed until moisture is sufficient.

(4) Suitable temperature also must play some rôle in the formation of the perithecia. Formation of perithecia took place only during the summer when the temperature was highest. Efforts to develop the perithecia from infected material during October and November gave negative results.

When the foregoing conditions were established as factors in the formation of perithecia, the following method of producing them was worked out and has yielded good results. The infected parts of the various cereals, including corn, such as stems, nodes, sheaths, heads, and ears, were gathered from the field and laid on the ground during July and August, 1918, then covered with a wire screen, moistened thoroughly, and covered with some dry grass and leaves to protect them from drying out. During the first and second weeks, masses of conidia were formed over the entire infected area of the various parts. Soon this extended even over the uninfected area. Before long all conidia germinated and no others were formed. During the third week the perithecia began to be formed. In three or four more weeks numerous perithecia were formed, most of them with matured ascospores.

The following is a record of one of the experiments for perfect-stage development:

June 28, 1918. Rye heads infected with *Gibberella saubinetii* were placed under screens so as to be exposed to the action of the weather. They were sprayed thoroughly with water and covered with dry grass to protect them from drying out.

July 16, 1918. First perithecia beginning to appear.

August 2, 1918. Numerous perithecia formed, but asci not yet fully developed.

August 21, 1918. All perithecia have ripe ascospores. Heads taken to the laboratory for study.

IN LABORATORY.—Infected wheat kernels, when placed in a pot filled with fine sand and only slightly covered with sand and kept moist at

room temperature, produced numerous perithecia on their exposed surfaces. These matured before the end of the fourth week from the time of sowing. As soon as the ascospores in the perithecia were found to be mature, the kernels were sifted from the sand and preserved in dry condition until needed for study or inoculation.

The development, in the laboratory, of perfect stages of those species of Fusarium which have a perfect stage was secured in the way originally described by Appel and Wollenweber and later extended by Wollenweber. It need only be emphasized that the perithecia of these fungi will rarely be formed until the transfers and cultural work are begun from what these authors call "normal" culture. Failure is bound to occur 95 times out of 100 before the culture which is to be used for development of the perfect stage is brought to this condition.

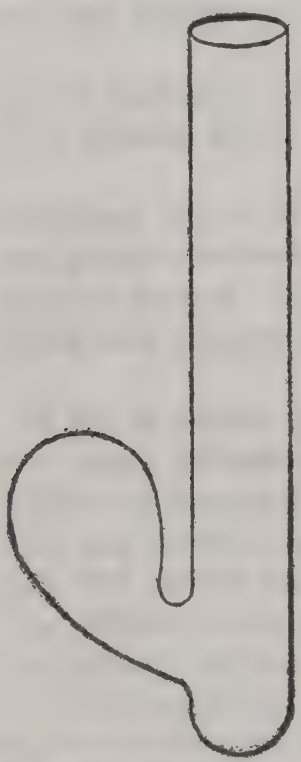


FIG. 2.—Special culture tube for maintaining moisture in culture. × ½.

Once the culture is in the proper condition, the next step consists in transferring it to media that are known to favor the development of the perithecia, such as stems of any kind, but especially those of *Melilotus alba*, bean pods, etc.

Care must be taken that the cultures on *Melilotus alba* stems or other media are kept uniformly moist until the perithecia are formed and the ascospores in them are ripe. The presence of certain bacteria in the cultures greatly favors the formation and proper development of the perfect forms of species of Fusarium. A certain bacterium which was found in a contaminated culture when added to cultures of Fusarium having perfect forms favored the formation of perithecia so much that practically 100 per cent of the cultures to which this organism was added developed numerous normal perithecia, while even under best conditions only a small number of the cultures to which this bacterium was not added produced perithecia. What this organism is and whether other bacteria can produce the same result are not known.

Heretofore the whole work of producing the perfect stage of any ascomycete in pure culture has been handicapped to a certain extent by the fact that the cultures of such fungi dried out long before the formation and ripening of the perithecia. The addition of water to the cultures from time to time exposes them to contamination and varies the amount of moisture in the culture considerably. To avoid this the writer designed a special culture tube. This consists of a common test tube, to the lower end of which is attached a bulb (fig. 2). When the bulb is filled with water it will drain into the test tube as rapidly as the

water from the test tube evaporates or is used by the fungus. Such a tube provides stem or potato plug cultures with uniform moisture for four or five months without being refilled. This is as long a period of time as is necessary for the formation of perithecia in any case. When stems are used they can be placed directly in the test tube so as to reach the bottom, but when potato plugs, bean pods, or other cultural substrata are used it is better to place some cotton on the bottom of the test tube so that the plugs will be just above the water level. Such test tubes are handled in very much the same way as common test tubes, except that more care should be taken in sterilizing them, since a sudden decrease in the pressure in the sterilizer is likely to force the water out of the bulb into the tube.

PATHOGENICITY

PREVIOUS INVESTIGATIONS

A large number of Fusarium species have been reported by various workers as attacking the cereal crops in one way or another. In a large number of cases the particular organisms have been wrongly identified or not identified at all. The true relation of the various Fusarium species to the different diseases on the cereal crops attributed to these species is even less understood than their taxonomy. Indeed, there are but few papers out of over 200 references in which proof of the pathogenicity and true relation of some of these organisms to certain cereal diseases is given. No papers except those most directly connected with the problem can be mentioned here.

Selby (*9*) considered *Gibberella saubinetii* and its conidial form which he, following Saccardo, called *Fusarium roseum*, as the cause of the blighting of wheat heads, but he failed to produce the disease by inoculating heads with the conidia and ascospores of this organism. In 1909, Selby and Manns (*11*) succeeded in producing blighting of wheat and oat heads by spraying them during moist weather with a suspension of conidia obtained by washing samples of wheat, barley, oats, emmer, and spelt. In this way they thought they obtained the conidia of *F. roseum* and its perfect form, *G. saubinetii*. It is very likely that it was the conidia of *G. saubinetii* that caused blighting of the heads in their experiment, but it is incorrect to suppose that conidia of only this species of Fusarium are found on samples of cereals. They also showed that pure cultures of *G. saubinetii* from various sources when added to sterile soil in which wheat and oats were sown caused severe rotting of the roots and killing of the young seedlings.

Schaffnit (*8*), studying the cause of what is known as "snowmold" in Europe, showed that while *Fusarium nivale* Ces., the conidial form of *Nectria* (later *Colonectria*) *graminicola*, is the primary cause of "snowmold" of the cereal crops in Europe, the following organisms are also more or less responsible for this disease: *F. culmorum* (*F. rubiginosum*),

F. herbarum (*F. metachroum*) (13), *F. didymium*, *F. avenaceum* (*F. subulatum*), and *F. lolii*. He showed also that *F. nivale* causes not only the snowmold but also rotting of the roots and killing of the young cereal seedlings. Later it causes footrot of the grown plants, usually following the wounding of the plants by insects or other agencies. *F. nivale* attacks the heads of the cereals during the period beginning at blossoming time and extending to the ripening of the crops and causes blighting. In this connection he distinguished between primary infection, which takes place before the ripening of the plants, and secondary infection, during the period of maturity and harvest. In the secondary infection he found that not only *F. nivale* but also less parasitic Fusarium species play an important rôle.

Naumov (5), studying the cause of cereal headblighting, which is reported to be severe throughout Russia, found that *Gibberella saubinetii* and *Fusarium avenaceum* (*F. subulatum*) are the cause of this disease in Russia and Siberia, the first being common in the southern and the second in the northern part of the country.

Studying the pathogenicity of these organisms, Naumov reported that:

(1) Infection of the soil will result in the blighting of heads of wheat and barley. How the organisms introduced into the soil under sterile conditions reach the heads of the plants where they cause blighting is not quite clear. Throughout the paper Naumov states that the mycelium of these Fusarium species is found in all parts of the plants, but it is not very clear whether infection in the roots and the lower parts of the plant proceeds up the stem, becoming systemic, or whether the various parts are infected separately by external infections. Though this view is not directly and plainly stated, in many cases the reader will be led to believe that Naumov considers the infection systemic and that it proceeds from the roots up to the heads, since in many places in this paper he speaks of finding the mycelium of these organisms in all the tissues of roots, stems, heads, leaves, and sheaths, but nowhere causing any anatomical changes.

(2) Spores or conidia of the causal organisms when on the seed, or naturally infected seed, can cause blighting of the seedlings.

(3) Conidia, ascospores, and mycelium of the organisms, when placed on normal young plants, with or without wounding, cause infection.

(4) Spraying the heads of wheat, rye, and oats with a water suspension of conidia of these organisms produced typical blighting of the infected heads as observed in nature.

(5) The results given under (4) were also obtained with ascospores of *Gibberella saubinetii*.

(6) These organisms can invade the tissues of the seed, straw, and heads of the cereal crops after ripening and harvesting if conditions are favorable.

EXPERIMENTAL RESULTS

ISOLATIONS.—In the vicinity of Madison, Wis., where the writer secured most of his material, *Gibberella saubinetii* is the most common and most important cause of the headblight of the cereals, and the writer believes this to be true throughout the country. The following Fusarium species were also isolated from blighted heads and other parts of the cereal plants: *Fusarium avenaceum*, 10 times—4 times from wheat heads from a field near Madison and 6 times from a single sample of 10 blighted spelt heads from Hawthorne, which is located in the extreme northwestern part of Wisconsin; *F. herbarum*, 8 times—3 times from blighted wheat heads from a lodged wheat field near Madison, Wis., and 5 times from corn stalks; *F. culmorum*, once from a blighted wheat head from Arlington, Va.; *F. culmorum* var. *leteius*, twice from blighted wheat heads from a lodged wheat field near Madison, Wis.; *F. arcuosporum*, 10 times—once from a blighted oat seedling and 9 times from barley heads left in the field late in the fall and cornstalks early in the spring; *F. scirpi*, four times from blighted wheat heads from a lodged wheat field and once from a blighted wheat head from one of the Experiment Station plots at Madison, Wis., which was badly overgrown with weeds; *F. solani*, once from a grown wheat plant showing footrot; *F. arthrosporioides*, 5 times—once from a blighted wheat head from a lodged wheat field and 4 times from blighted barley heads; *F. redolens*, 3 times—once from a discolored rye stem near a node, once from a blighted wheat head from Knoxville, Tenn., and the third time from a blighted barley head from a weed-overgrown plot in the Experiment Station field, Madison, Wis.

On the other hand, *Gibberella saubinetii* was identified by the writer on over 2,000 blighted wheat, barley, rye, oat, and spelt heads from various parts of the following States: Wisconsin, Illinois, Minnesota, Indiana, Maryland, Kentucky, Ohio, Virginia, West Virginia, South Carolina, Georgia, Alabama, North Dakota, and Michigan. This shows that, from the standpoint of headblight of the cereal crops, *G. saubinetii* is the most important organism.

All species of Fusarium given here, including *Gibberella saubinetii*, were isolated originally by poured-plate dilution of conidia from distinctly blighted wheat heads. During the course of the work, however, some of these species were often isolated from blighted rye, barley, and oat heads, or stems, and from sheath, shank, root, and node rots of corn, or in a few cases from other hosts. The organisms attacking the cereal crops above the ground produce numerous conidia over the infected area. The conidia so produced are often normal and uniform in size and shape, and the trained student will not only have no difficulty in separating the various species before he has grown them under artificial conditions but he will be able also to determine in a general way the various species, at least the various sections to which they belong.

In order to prove that the Fusarium conidia produced on a blighted wheat head are the conidia of the causal organism and not of a secondary organism which has followed the first, parts of a large number of blighted wheat heads were washed in distilled water to moisten them and then disinfected by dipping them in 1 to 1,000 mercuric chlorid solution ($HgCl_2$) for two minutes. After this they were rinsed in distilled water and then transferred with a sterile needle to cooled poured plates of a suitable medium. In all cases only one organism was isolated from each blighted head, and this was in all cases the same as the one obtained from the conidia on this head. This is so true of the Fusarium organisms causing headblight that the causal organism upon a clean, undiscolored Fusarium-blighted head may almost surely, and even without microscopic examination, be described as one and pure. In rare cases the blighted heads may also be smutted, rusted, or brown spotted and discolored; and in such cases, of course, more than one organism may be found on a head. Such heads were discarded and never used for study or isolation.

Plain water agar [1] was used for diluting the conidia and for pouring the plates. After 12 to 24 hours the plates were examined microscopically, and single, germinating conidia were marked on the plate; then with a sterile needle made for the purpose they were transferred to test tubes containing suitable medium, usually hard oatmeal agar. In all cases five single, germinating conidia were transferred, with only one to each test tube. This was done to make sure that there was not more than one species of Fusarium present. Except in rare cases when some of the test tubes were contaminated during the manipulation with foreign organisms such as Penicillium or bacteria, all five test tubes yielded the same species. To make certain, however, that the cultures were free from bacteria they were transferred to plates, and second transfers were made from the margins of the plate colonies. The pure cultures so obtained were used as stock cultures for further study.

Inoculation work.—In this paper only the results of inoculation with *Gibberella saubinetii* are given. The writer was able to produce blighting of heads of wheat and rye by inoculation with several of the species mentioned above and was able to produce more or less severe seedling-blight by inoculation with nearly all of them, but the conditions under which these species become pathogenic are not yet well understood.

Seed and soil inoculation.—A number of methods have been used in artificially infesting soil with species of Fusarium. Most of them consist in growing the particular organism on a suitable medium and then introducing the whole culture into sterilized soil. Such a method is very good, except that it is an artificial one which does not reproduce

[1] One liter of distilled water and 25 gm. of bacto-agar.

the conditions that actually exist in nature. It introduces into the soil various substances, toxins perhaps, which may have some effect upon the final results. In order to avoid this and to make conditions in the greenhouse as natural as possible, only conidia were employed for inoculation of the soils used for testing the pathogenicity of *Gibberella saubinetii* on young seedlings. Practically all Fusarium species when grown under proper conditions will produce large masses of conidia, which can be gathered from the substratum with a flat needle, free from any conidiophores or mycelial hyphæ, and suspended in a test tube or flask of sterile distilled water. If the conidia are not abundant, a fairly heavy conidial suspension may be obtained by washing the culture with sterile distilled water and straining the water through sterile cheesecloth. Suspensions of conidia thus obtained were used for inoculating the seed by dipping the seed into it for a few minutes. Spore suspensions thus obtained were used for artificially infesting sterilized soil by pouring part of the suspension upon the soil in each of the pots and mixing it with the upper layer of soil. By this method only a comparatively small number of conidia and only a negligible amount of foreign matter were introduced into the soil.

In all the soil experiments the soil used was sterilized in pots in an autoclave for 1 hour at 15 pounds pressure. All the seed used for sowing was placed for several minutes in a weak solution of saponin [1] and shaken hard, the object being to moisten the seed thoroughly and to remove all air bubbles adhering to it. The seed was then soaked for 30 minutes in 1 to 1,000 mercuric chlorid solution. Seeds so treated proved to be perfectly sterile on the outside. However, the fungi present in their internal tissues are not affected by this treatment. For this reason, only seeds that were comparatively free from such fungi and healthy in appearance were used for experimental purposes.

Throughout the work 6-inch and 12-inch pots and garden soil were used for sowing the seed. In each case two pots were planted with infested soil or seed, and one pot was sown as a control. Each experiment was repeated several times.

Seed of wheat, rye, barley, and oats naturally or artificially infected with *Gibberella saubinetii*, or planted on sterile garden soil artificially infested with this organism, showed a decrease in germination. In the case of the seed naturally infected, the decrease in percentage of germination is greater and is variable, depending on the degree of infection and percentage of seed infected. This may vary from 2 or 3 per cent to as high as 50 per cent. Artificially infected seed or seed sown on infested soil also shows a lower percentage of germination than the controls similarly planted. Here, too, percentage of germination depends on the kind and condition of the seed. It may vary from 0 to as high as

[1] One hundred cc. of 50 per cent alcohol and 1 gm. of saponin.

15 per cent. Good, healthy, plump seed may show no decrease in germination, while weak and shriveled seed may show considerable decrease in germination.

Gibberella saubinetii, besides preventing some of the seeds from germinating, attacked from 10 to 40 per cent of the young seedlings, causing rotting and browning of their roots, bases, and sheaths (Pl. 3, A). A number of the plants so attacked, usually few under normal conditions, rot and die before reaching the surface of the soil. Others wilt and die after reaching the surface, while the large majority recover almost entirely and attain practically normal development. Over 20 spring-wheat plants which showed marked rotting and browning of the roots and bases caused by this organism while they were grown on sterilized soil from infected seed in pots out of doors, when transplanted to the pathological garden recovered rapidly and reached full development, producing heads as normal as those on the control plants. Only 2 of the plants so transplanted wilted shortly after the transplanting, and the writer is inclined to attribute the wilting more to the transplanting than to the parasitism of the organism. This fact shows that, although *G. saubinetii* when present on the seed will infect many of the seedlings, it is not able to injure them materially unless the plants are growing under extremely unfavorable conditions, as was the case with the plants shown in Plate 2, B. In this case, the experiment was conducted during February, 1918, at a time when there was a minimum of sunlight in the greenhouse and when all the greenhouse plants were consequently weakened. The results of the experiment are summarized in Table I.

TABLE I.—*Average results of two inoculation experiments on each of 2 wheat samples, sample 1 consisting of hand-picked, healthy, plump kernels, and sample 2 consisting of hand-picked, healthy, but average kernels sown May 23, 1919, in pots kept out of doors*

Sample No.	Number of kernels.	Germination.	Number of healthy plants.	Number of plants showing rotting of roots and bases.	Number of killed plants.
		Per cent.			
1	Control, 100	91	89	2	0
	Inoculated, 100	90	75	15	2
2	Control, 100	76	71	5	0
	Inoculated, 100	69	42	27	6

While it was shown by numerous experiments that *Gibberella saubinetii* is able to decrease the percentage of germination of wheat, rye, barley, and oats and to cause rotting and browning of the roots and bases of some of the seedlings and even to cause wilting and dying of others, it was also noticed that this varied considerably from time to time and that some factors like light, temperature, moisture, and soil conditions have much to do with the degree and severity of infection.

Winter wheat, disinfected as described above, artificially inoculated with conidia of *Gibberella saubinetii*, and sown October 20, 1918, in five 12-inch pots of sterile soil with 10 kernels in each pot, was left in the greenhouse for 15 days and then taken out of doors, where it remained till July, 1919. A similar series of spring wheat similarly treated was sown on April 21 in pots of the same size but was left out of doors from the time of planting. Two pots sown with similarly treated but uninoculated seed were used as controls for each of the two series. In both series the plants recovered rapidly from the primary attack and grew normally, giving plants which were apparently normal, except that their bases and roots were slightly rotted and browned. With the coming of dry weather during the second half of June this rotting and browning of the roots and especially of the bases was intensified somewhat, and the plants began to wilt suddenly. In the field, wilting usually takes place at the time of heading or shortly after. The general symptoms accompanying wilting of fully developed plants are somewhat similar to those described for the footrot of the cereals in Europe and for "take-all" in Australia. *G. saubinetii* was isolated from the browned and rotted bases of the wilted plants in the foregoing experiments, as well as from those of some of the similarly wilted plants in the field.

HEAD INOCULATION.—While much work must be done before the nature and exact importance of the parasitism of *Gibberella saubinetii* on the underground portions of the cereal crops and the factors influencing or controlling it are fully understood, the question of headblighting due to this organism is much easier to follow and is, therefore, better understood.

The methods used in testing the pathogenicity of *Gibberella saubinetii* on wheat, rye, barley, oats, spelt, brome grass, quack grass, and timothy are very simple. They consist in producing a heavy suspension of conidia, either from heads already infected or from pure cultures, and spraying it by means of a small atomizer on a number of heads, usually 10, of the various hosts mentioned above when they are in the proper condition for infection. This method is successful when the weather is moist and cloudy. In dry weather this method will give either no results or only a very small percentage of infection. Certain results can be obtained only when the infected heads are in some way kept moist for at least three days after inoculation, and even this method will not give good results during extremely dry and hot weather. In the work described above the heads were kept moist by placing some moist absorbent cotton around the stems of a group of heads, then covering both the heads and the bundle of cotton around their stems with a glassine bag. The open end of the bag was tied around the stems just below the bundle of cotton. The heads so treated were heavy and required support. For this reason, garden stakes 5 or 6 feet tall were driven into the ground near the plants, and the bags covering the heads were tied loosely to them. The moist cotton inside of the bag kept the air comparatively moist and created

the condition desirable for successful infection. Since the glassine bags were transparent, the heads were not seriously deprived of sunlight. When the weather was very dry and warm the bags had to be opened and the cotton again moistened to saturation. All controls were treated in the same way as inoculated plants, except that they were sprayed with water to which no spores had been added.

Since *Gibberella saubinetii* usually produces very few conidia in culture, and since large quantities of spores were required for inoculations, it was necessary to contaminate the cultures purposely with a certain bacterium which has been found to bring about a great increase in sporulation. In this way large quantities of spores could always be obtained. The bacterium has not been identified, and the nature of its effect upon cultures of *G. saubinetii* is not known. Further study of this relationship is planned for the future.

The employment of such conidia for inoculation naturally raises the question whether the bacterium present has some effect on the pathogenicity of *Gibberella saubinetii* or whether it itself is pathogenic on wheat. In order to establish this, numerous wheat heads were inoculated at the same time with pure *G. saubinetti* conidia and others with a suspension of a pure culture of the unidentified bacterium. In all cases the heads inoculated with *G. saubinetii* conidia became blighted, while all heads inoculated with the bacterium suspension remained perfectly free from blighting or other injury. This shows that the bacterium favoring the sporulation and perithecia formation of *G. saubinetii*, as mentioned before, is not pathogenic on the wheat heads and has no effect upon the pathogenicity of *G. saubinetii*.

Wheat, spelt, rye, barley, and oat heads, as well as heads of *Agropyron repens* when inoculated with a conidial suspension or an ascospore suspension of *Gibberella saubinetii* became blighted. The blighting of *A. repens* proceeded exactly as observed in nature. In over 100 inoculation experiments in which over 3,000 heads of the various cereals, mostly wheat heads, were concerned, some infections always resulted. The number of blighted heads in each experiment varied from over 50 per cent to 100 per cent. In the majority of the experiments, all inoculated heads became infected and typically blighted. On many of these heads conidia were formed, and on some even the perithecia of *G. saubinetii* developed before the harvesting of the plants.

The inoculation experiments gave positive results from the time of blossoming till the latter part of the dough stage. Inoculation made before the first and after the second stage gave either negative or very doubtful results.

PERIOD OF INCUBATION

ON SEEDLINGS.—The period which elapses between the inoculation and the time the first symptoms of attack on the seedling roots appear varies so much that no definite incubation period can be given. It varies

considerably with the condition of the seed used. When light, shriveled seed is sown on infested soil, or when such seed is inoculated by being dipped in a suspension of conidia and then sown on sterile soil, the seedlings will succumb to the attack of the parasite much more rapidly than when healthy seed is used. Abundant watering of the plants also increases to some extent the rapidity of the attack.

In general, under greenhouse conditions, the first symptoms of root infection appear not earlier than the seventh day after sowing. Infection is usually abundant after the fourteenth day. When naturally infected seeds have been used on sterile soil the symptoms of root infection may appear even before the seventh day.

ON HEADS.—In head infection there is much less variation in the incubation period. In damp weather, the period that elapses between inoculation and the appearance of the first symptoms (water-soaking) varies from three to six days. In dry weather, symptoms of infection may not appear until after the first rain, or if heavy dew falls during the night and lasts for the greater part of the forenoon, symptoms of infection may appear from five to eight days later.

The rapidity with which the blight infection spreads from the point of infection to the rest of the head varies greatly. It varies considerably with different individuals and depends much upon the kind of weather. On healthy, vigorous, and more succulent plants the infection spreads much more rapidly than on plants of average vigor. Moist and cloudy weather, followed by warm and clear weather, greatly accelerates the rapidity of infection and killing, yet even under such conditions the infection may be restricted on many heads to a single spikelet, the rest of the head remaining healthy and developing perfectly normal, plump kernels.

For the study of the rapidity of the spread of the disease from the point of infection, heads showing primary infection were located daily and marked with tags so that they could be located again. Heads so tagged were examined every two or three days and the changes recorded. In this way the effect of the various factors affecting the rapidity of blight infection and killing were studied. The following are typical records of some infected heads, made in 1918:

N 1009, July 11, 1 spikelet infected. Infection at base of head.
July 14, 4 spikelets infected.
July 17, whole head killed.
N 101, July 11, 5 spikelets infected. Infection at middle.
July 14, 8 spikelets infected.
July 17, whole head killed.
N 1038, July 9, third spikelet from bottom infected.
July 14, 4 spikelets infected.
July 17, Whole head killed.
N 1039, July 9, 1 spikelet infected. Infection at middle.
July 14, 4 spikelets infected.

N 1039, July 17, 12 spikelets infected.
July 24, whole head killed.
N 1156, July 11, uppermost spikelet infected.
July 24, 1 spikelet infected. Plant almost ripe. Infected spikelet covered with Fusarium conidia.

There has been considerable discussion as to whether the headblighting of the cereal crops caused by *Gibberella saubinetii* and some other Fusarium species is the result of a systemic invasion of the host plants by these organisms. Naumov (5), as stated before, considers the invasion systemic. He finds the mycelium of the fungus in all parts of the plants and even in plants showing no blighting of the heads. He showed that infection of the heads can also take place externally.

Since there is uncertainty in determining from its appearance the kind and nature of any mycelium that may be present in the tissues of the cereal plants, it was thought that the easiest and only reliable way to show whether certain plants carry in their tissues the mycelium of *Gibberella saubinetii* or any other Fusarium species would be to plate out portions of such plants on some suitable artificial medium on which the organisms are known to thrive well. If they are present in the tissues of the plated plant they are sure to appear on the plates.

Wheat and rye plants with blighted heads where the infection from the heads has extended to the upper part of the upper internode, as previously described in this paper, were used for plating. Such peduncles were cut in portions 1 inch long, beginning from the end next to the blighted heads. These portions were disinfected on the outside by dipping them in 1 to 1,000 mercuric chlorid for two minutes. They were then rinsed in sterile distilled water and plated in order on hard potato agar. In all cases colonies of *Gibberella saubinetii* were formed over the portion next to the infected head and in some cases over the adjoining portion. The portion of the peduncle which was farthest from the head and perfectly green and free from discoloration never developed any fungous growth (Pl. 3, B). This shows very conclusively (1) that the infection on the cereal heads is local, and (2) that it proceeds from the head down and not from the roots up.

WEATHER CONDITIONS IN RELATION TO HEAD INFECTION

Weather is one of the important factors for the successful parasitism of *Gibberella saubinetii* and the various Fusarium species on the cereal crops. Indeed, it is the limiting factor for the occurrence of head-blight under certain conditions, and its importance was noticed early by students of the subject. Dry weather with slight winds during and after the period of blossoming and extending well toward the dough stage will practically eliminate blight infection though all the other necessary conditions may be present. It was observed in many cases that in fields where there have been only few blighted heads before the

coming of rains and cloudy weather there was a marked increase in the number of blighted heads only a week after the rain. This was shown very plainly in experiment 22, one of the inoculation experiments in 1918.

At 7 o'clock in the afternoon, July 2, 1918, 60 wheat heads in one of the Wisconsin Experiment Station plots were sprayed with a suspension of *Gibberella saubinetii* ascospores and left uncovered.

On July 8, 1918, 12 heads, or 20 per cent, showed signs of first infection. Several days later there came a slight rain and the sky was cloudy for over a day. By the twentieth of the same month 28 heads, or 45 per cent, showed symptoms of blighting.

On the other hand, an experiment, which differed from the foregoing only in that the heads were kept moist artificially (see inoculation experiments, p. 25), showed 70 per cent infection on July 7, 1918. The number of the infected heads did not increase after the rainy and cloudy weather that followed. All controls in both experiments remained healthy. This case, which is one of several, shows that in the absence of proper weather conditions there is much less infection than when the weather is favorable. In experiment 20, in which the heads were kept moist, all the heads that were successfully infected showed infection within six days, and the coming of rain in this experiment did not increase the number of infected heads.

Not only does rainy and cloudy weather favor blight infection but it is also necessary for spore production, as already pointed out in this paper.

CULTURAL CONDITIONS IN RELATION TO HEADBLIGHT

Even though they were well developed and still apparently healthy and normal, the plants which were in shady places or overgrown by weeds were attacked by headblight and noderot to a much greater extent and by a greater number of the species of Fusarium than were plants which had a normal amount of sunlight. This was especially evident in one of the Wisconsin Experiment Station plots where a small area sown with barley and wheat was allowed to be overgrown by weeds. The blight infection on this plot was so abundant that in some small areas practically all the plants were infected. In general, the whole field had an average of 10 per cent of infection as compared with 5 per cent from neighboring clean fields. Another interesting fact was that nine different species of Fusarium, two of which have perfect stages, were isolated from blighted heads gathered from this small plot covering not over 200 square yards. *Gibberella saubinetii* was the most common and most destructive species.

Lodging of the fields also gives a marked increase of headblight infection. This was brought out especially well in a wheat field located two miles northeast of Madison, Wis., where the head infection among the

standing plants even in the worst-infected portions of the field never exceeded 15 per cent, while in the lodged portions of the field the head infection was, in some small areas, as high as 100 per cent. Considering that the field was not over two acres in extent, that the inoculum of *Gibberella saubinetii*, which was responsible for over 90 per cent of the infections in this field, was very uniformly distributed throughout the field, and that there were no other explanations for this great difference in degree of infection between the lodged and the standing plants, the effect of lodging on the prevalence of headblight infection becomes more striking.

VARIETIES IN RELATION TO THE DISEASE

During the summer of 1918 more than 30 varieties of wheat, both winter and spring, were grown by the Department of Agronomy, University of Wisconsin, on the University farm, and all were attacked more or less by headblight. There was marked difference between them in the degree of infection, but no variety was entirely free. As will be seen from the list given in Table II, among the varieties examined were representatives of types having very different morphological characters, from those which have very fine and succulent chaff to those which have hairy or very hard chaff.

Since the winter varieties examined were badly winter-killed, no significant count could be taken which would indicate their relative susceptibility to headblight. The spring varieties, on the other hand, were in very good condition and uniform throughout the series of plots.

The 15 spring-wheat varieties were sown in small plots of the same size, the plots being in one series which extended across the whole field. The whole series of varieties was repeated so that the variety planted on the first plot was repeated on the sixteenth plot, the variety planted on the second plot was repeated on the seventeenth plot, and so on. The plants in each plot were examined carefully and the blighted heads counted. The number of blighted heads of each variety in the two series was in many cases exactly the same. If there was a difference, it did not amount to more than two or three heads. The results are given in Table II.

These results, while not convincing, are very interesting, especially when we consider that all plots had the same preparation and cultivation, the same preceding crop, were on the same piece of land, that all varieties, while not in exactly the same stage of development, were in a stage in which they were susceptible to blight, and that the degree of infection of a certain variety was the same in the two series located a considerable distance apart.

One may suspect that the relative amount of infection of the seed used for sowing is the cause both of the difference of infection between different varieties and of the uniformity in degree of infection of the same

variety in both series. While this seems possible, it does not seem probable in this case. The plots were small and only 2 feet apart, so that if some plots were more heavily infected because of the more heavily infected seed sown on them the inoculum from them could easily have served for the plants in the neighboring plots only 2 feet away. The plot with the variety Preston × Kubanka cross (Wisconsin 101), which had 22 blighted heads, was between plots that had only 1 and 3 blighted heads, respectively.

TABLE II.—*Averages of actual counts of blighted wheat heads in two series of different varieties, arranged according to degree of infection*

Variety.	Wisconsin No.	Number of heads blighted.
Preston × Kubanka cross	101	22
Red Fife	46	20
Red Fife selection E. G. D. 9171	75	20
Marquis	50	15
Marquis selection	48	16
Pedigree Marquis	29	12
Red Fife selection	74	9
Fife, Minn. 163	Pedigree 34	9
Spring Velvet Chaff	60	7
Haynes Bluestem × Kubanka cross	102	7
Spring-wheat selection	76	3
Bluestem	Pedigree 35	3
Bluestem	Pedigree 36	1
Spring-wheat selection	98	1

The differences between varieties in susceptibility to blight was brought out more plainly in a field where two spring-wheat varieties, Marquis and durum, were sown side by side on the same piece of land, following corn. The infection of the Marquis wheat where the plants were standing was less than 1 per cent and from 10 to 15 per cent among the lodged plants, while the infection among the standing durum plants was from 9 to 10 per cent and as high as 100 per cent among the lodged plants.

Throughout the field there were numerous cornstalks with perithecia containing viable spores of *Gibberella saubinetii* and other parasitic species of Gibberella, as well as numerous viable conidia of several blight-causing Fusarium species. While we can doubt the result obtained with various varieties on the University plots, the results obtained on this field indicate clearly the existence of a difference in varietal susceptibility to head-blight. Further observations and experiments in this direction will, no doubt, be of great importance.

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A set of 509 plates prepared to accompany this work, but not published, is on file in the Office of Cotton and Truck Crop Disease Investigations of the United States Department of Agriculture.

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PLATE 1

Gibberella saubinetii:

Blighted ("scabbed") wheat heads. Control plant on the right of others, showing gradation of blighting to completely blighted head on the extreme left.



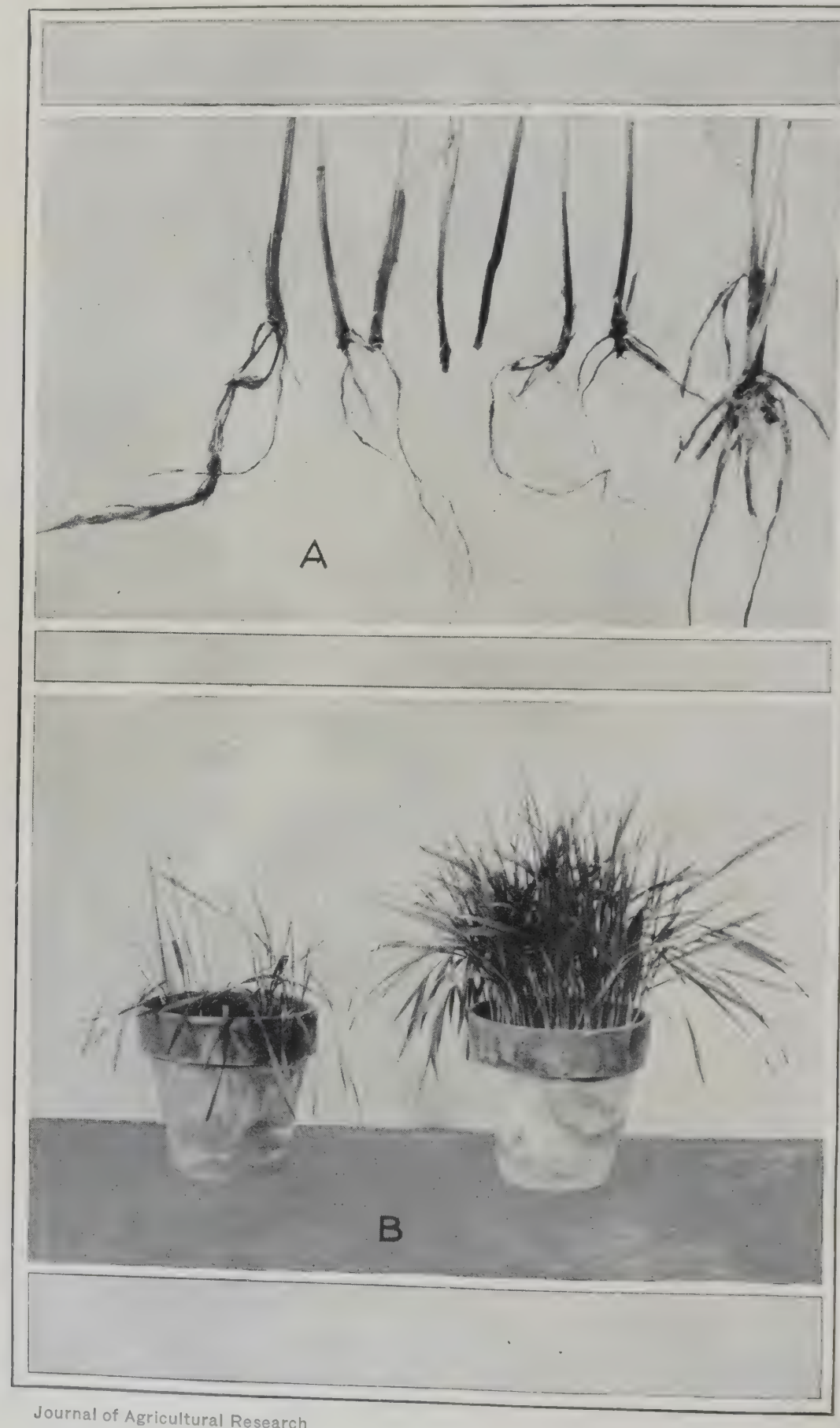

A
B

PLATE 2

Gibberella saubinetii:

A.—Footrot of wheat caused by Fusarium. The plants at the left were taken from soil which had been inoculated with *G. saubinetii*. The control plant at the right gives the comparative size of the normal wheat root system.

B.—Seedling-blight of wheat caused by *G. saubinetii*. The seed in the pot on the left was inoculated with *G. saubinetii* conidia before planting. The control pot on the left was planted with clean seed. Germination was reduced, and many of the seedlings were killed.

PLATE 3

A.—Fusarium seedling-blight. The normal plant is on the left. The other five show the gradations in blighting caused by *Gibberella saubinetii*.

B.—Tissue invaded by *G. saubinetii* in causing the headblight of wheat. Each group includes the four consecutive sections which, after surface sterilization, were cut from the upper internode of a wheat culm having a blighted head, the left segment in each group being the upper. These were then incubated on agar plates. Note that only the sections nearest the head were invaded by the fungus.


B
A


A
B

PLATE 4

Gibberella saubinetii:

A.—Kernels blighted and shriveled by Fusarium-blight. Wheat kernels above are typical of Fusarium-blight. They are shriveled and much lighter than the normal kernels below.

B.—Perithecia development of *G. saubinetii* on an infected wheat head.

CAUSE OF LIME-INDUCED CHLOROSIS AND AVAILABILITY OF IRON IN THE SOIL

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CAUSE OF LIME-INDUCED CHLOROSIS

INTRODUCTION

Some years ago a study was made of a chlorosis of pineapples that occurred on certain soils in Porto Rico (*12*).[1] The particular type of chlorosis was confined to calcareous soils and seemed to be induced by a disturbance in the mineral nutrition of the plant. This disturbance appeared to consist in a lack of iron in the plant ash or in a diminished amount of iron combined with an increased amount of lime. Considerable work has since been carried on to determine more exactly the manner in which carbonate of lime in the soil induces chlorosis in the plant. The work comprises a number of direct experiments on the cause and cure of chlorosis as well as general studies in plant nutrition undertaken to gain information necessary for interpreting results obtained in the experiments on chlorosis. Since the more general work on plant nutrition has been published elsewhere, only the results will be referred to here.

In the following pages the more important facts already established concerning the cause of lime-induced chlorosis are given, together with a full report of certain experiments on this subject hitherto unpublished.

EVIDENCE THAT CARBONATE OF LIME MAY INDUCE CHLOROSIS

Evidence that carbonate of lime produces chlorosis in certain plants naturally falls into two classes, the results of soil surveys and the results of direct tests with natural or artificial calcareous soils. These two classes of evidence will be considered separately.

RESULTS OF SOIL SURVEYS

ECOLOGICAL STUDIES OF CALCIPHILOUS AND CALCIFUGOUS PLANTS.—Under the heading of soil surveys, reference should be made to the extensive literature on calciphilous and calcifugous plants. This literature, of which Roux (*39*) gives a complete bibliography up to 1900, consists chiefly of observations concerning the confinement of certain plants to calcareous or noncalcareous soils. While most of these

[1] Reference is made by number (italic) to "Literature cited," p. 59–61.

observations do not deal directly with chlorosis, all are related to this subject, since calcifugous plants are often chlorotic on calcareous soils and since an exposition of the causes of chlorosis may afford an explanation of the calcifugous character of some plants.

There are a few plants which are very generally classed as calcifugous. Among these are the following: Maritime pine (*Pinus pinaster*) (*9*) chestnut (*Castanea vesca*), blueberry (Vaccinium), yellow and blue lupines (*Lupinus luteus* and *L. angustifolius*), certain species of sphagnum moss, etc. Cases have been recorded, however, where some plants generally considered calcifugous have been found growing on calcareous soils (*7*).

Probably the unsuitability of calcareous soils for certain plants is due not to carbonate of lime itself but to some soil characteristic usually associated with carbonate of lime. This being so, calcifugous plants might occur on certain calcareous soils provided some factor were operating to counteract the inhibiting characteristic usually associated with carbonate of lime.

STUDIES OF CHLOROTIC PLANTS.—Besides the soil surveys of calcifugous plants, there are several soil surveys which deal directly with the appearance of chlorosis in cultivated plants.

A case that has been the subject of much study is that of European grapes grafted on certain American stocks. When these were introduced on the calcareous soils of France and Germany they became chlorotic. Several soil surveys and many observations prove that the chlorosis is confined to calcareous soils and that there are varietal differences among grapes with respect to their resistance to lime (*22, 30, 33, 39 Viala and Ravaz, 45*). The accumulated data do not show, however, that all soils containing more than a certain percentage of carbonate of lime produce chlorosis in these varieties of grapes.

The chlorosis and failure of chestnut trees on most soils containing more than 3 per cent of carbonate of lime has been well established through soil surveys and through observations by Fliche and Grandeau (*10*), Piccioli (*36*), Vallot (*44*), and others. Vallot (*44, p. 202*) states that Dr. Bonnet reported that the chestnut failed to grow in a calcareous soil of Dijon, but when it was grafted on an oak it grew superbly.

That yellow and blue lupines and serradella become chlorotic when planted on calcareous soils is common knowledge in the calcareous districts of France and Germany, 2 per cent of carbonate of lime usually being sufficient to affect these plants.

A soil survey in Porto Rico showed that a chlorosis of pineapples was confined to the calcareous soils (*12, p. 8–18*). The only calcareous soils not producing chlorotic pineapples on which data could be obtained were some from the Florida Keys. These contained an exceptional amount of organic matter.

A chlorosis of sugar cane in Porto Rico was also found to be confined to calcareous soils, although very many calcareous soils did not induce

chlorosis. Green cane was found growing on a soil containing 76.70 per cent calcium carbonate (*19*).

Pears have frequently been reported as showing chlorosis on calcareous soils (*4*, *6*, *29*, *38*).

Instances have been noted where a great many other plants have become chlorotic on calcareous soils (*24*). Many of these cases are doubtless more or less exceptional, since some of the plants do not become chlorotic on most calcareous soils. Roux (*39*), without attempting a complete compilation, mentions some 50 genera and species of cultivated plants, ranging from mosses and orchids to maples and citrus trees, which have shown chlorosis when planted on soils containing calcium carbonate.

The results of the soil surveys and field observations seem to demonstrate conclusively that this type of chlorosis is confined under field conditions to calcareous soils. Probably no one species of plant, however, becomes chlorotic on all soils containing more than a certain percentage of calcium carbonate. Some plants are much more sensitive to carbonate of lime than others—that is, they become chlorotic on soils with lower lime contents and are less frequently found growing normally on limy soils.

The fact that plants very subject to chlorosis have been found in a few instances growing normally on markedly calcareous soils shows that the ability of calcareous soils to induce chlorosis does not depend entirely on the percentage of carbonate of lime in the soil. This fact also lends credence to the idea that it is not the carbonate of lime itself that induces chlorosis but some condition usually associated with the presence of carbonate of lime.

RESULTS OF VEGETATIVE EXPERIMENTS IN WHICH CHLOROSIS WAS PRODUCED BY NATURAL OR ARTIFICIAL CALCAREOUS SOILS

Compared with the mass of observations on the natural occurrence of chlorosis, there has been little reported in regard to inducing chlorosis by the use of calcium carbonate or in regard to direct tests of calcifugous plants in calcareous soils. There have been several vegetative experiments with yellow and blue lupines, however, where the addition of carbonate of lime to the soils caused a marked depression in growth and, in some cases at least, induced chlorosis. Concordant, positive results were secured by Heinrich (*23*), Meyer (*32*), Pfeiffer, and Blanck (*35*), the Agricultural Chemical Experiment Station at Breslau (*2*), Creydt (*5*), and Roux (*39*, *p. 147–185*).

Büsgen (*3*) grew the calcifugous broom (*Sarothamnus scoparius*), foxglove (*Digitalis purpurea*), and heather (*Calluna vulgaris*) in artificial calcareous and noncalcareous soils. The growth of all three plants was moderately to greatly depressed in the calcareous soil, although only broom was mentioned as showing chlorosis.

Roux (*39, p. 147*) grew some 20 species of calcifugous plants in calcareous soils. All species made diminished growth and became chlorotic in certain calcareous soils, while none showed chlorosis in the noncalcareous soil.

Piccioli (*36*) planted many varieties of chestnuts, together with Sarothamnus, Calluna, and Pteris, on soils with different additions of carbonate of lime. Most plants eventually died on the soil containing 12 per cent calcium carbonate.

Experiments at this Station showed that the mere addition of carbonate of lime to soils which normally produced green pineapples (*12, p. 20*) or rice plants (*13, p. 30*) caused the soils to produce chlorotic plants.

The preceding experiments seem to afford direct proof of the conclusions derived from field observations and from soil surveys that a chlorosis of some plants is caused by, or associated with, the presence of carbonate of lime in the soil.

MANNER IN WHICH CARBONATE OF LIME IN THE SOIL INDUCES CHLOROSIS IN THE PLANT

While it is quite generally conceded that carbonate of lime may induce a chlorosis in certain plants, there is a great diversity of ideas regarding the way the chlorosis is brought about. There are several classes of evidence or kinds of data on which conclusions concerning the nature of lime-induced chlorosis are based. These different kinds of evidence will be considered under the following heads: Evidence from analyses of plant ashes, effect of application of iron salts, effect of other lime compounds in inducing chlorosis, and effect of an alkaline reaction in inducing chlorosis.

RESULTS OF ASH ANALYSES OF PLANTS

In their work on the chlorosis of the chestnut and maritime pine Fliche and Grandeau (*9, 10*) analyzed leaves and branches of green and chlorotic trees. They concluded that the chlorosis and diminished growth of the trees on the calcareous soils were the result of an undue absorption of lime and a diminished absorption of other elements, notably potash and iron.

Schulze (*42*) analyzed the wood and leaves of green and chlorotic grapevines,[1] determining only lime, magnesia, potash, and soda. Compared with the green plants, the chlorotic ones had about one-half as much potash and soda and slightly more lime and magnesia in the ash.

Büsgen (*3*) analyzed the broom plants grown by him in calcareous and noncalcareous soils to determine lime and potash. The chlorotic and

[1] Analyses by Mach and Kurmann (*31*) are often quoted in this connection. The results probably have no bearing on this subject, however, as the chlorosis of their specimens seems to have been caused by too much moisture or poor drainage.

green plants from the two soils had almost equal percentages of lime and potash in the ash, the percentage of total ash in the dry substance being higher in the chlorotic plants.

Numerous ash analyses were made at this Station from chlorotic and green pineapple plants grown in soils with and without carbonate of lime (*12*). Compared with the green plants, the chlorotic ones in the calcareous soils contained more lime and less iron in the ash; differences in other ash constituents were slight or inconstant, potash as a rule being fully as high in the chlorotic plants as in the green ones.

Green and chlorotic rice plants were also analyzed at different ages for their mineral constituents (*13*). In the case of rice grown 25 days, the chlorotic plants from the calcareous soils contained much more lime, less iron, and equal or greater percentages of potash in the ash than the green plants from the soil containing no carbonate of lime; but in the case of green and chlorotic rice of 84, 102, and 129 days' growth, the only constant difference in the ash of the two kinds of plants was a greater percentage of lime in the chlorotic plants. These analyses and a special study showed that the percentage of iron in the ash of rice diminished very markedly as the plants became more mature (*15*). Since plants affected with chlorosis matured much more slowly than normal plants, probably the iron contents of the 84-, 102-, and 129-day samples were affected more by the different maturities of the plants than by the character of the soils.

Four pairs of samples of green and chlorotic sugar-cane leaves were analyzed for their ash constituents. The leaves were selected from canes which were of the same size and age and which were growing on the same calcareous soil. In each case the chlorotic leaves had a distinctly lower percentage of iron in the ash than the corresponding green leaves (*19*).[1]

A summary of the evidence from ash analyses in regard to the cause of lime-induced chlorosis is as follows: Lime was determined in all seven species of plants analyzed by the different investigators, and in five cases it appeared that an excessive absorption of this element might be a cause of chlorosis; in two cases it appeared that it was not. Potash was determined in six of the different plants, and in only three cases did it appear that a lack of potash might be a cause of chlorosis. Iron was determined in five of the plants, and in all five cases it appeared that the chlorosis might be due to a deficiency of this element.

The weight of the evidence from the ash analyses seems to be that a deficiency of iron in the ash is at least one cause of the chlorosis and that possibly an excess of lime is also a cause. Against this conclusion there is the opinion of many physiologists, as Euler (*8*), Jost (*28*), and Sorauer

[1] In a fifth comparison, leaves of green, slightly chlorotic, and chlorotic cane were analyzed, the canes being of equal age but of markedly different size when grown on calcareous and noncalcareous soils. The chlorotic leaves contained very slightly more iron than the green leaves. In this case, it is believed that the maturities of the plants and the different ages of the leaves were the chief factors influencing the iron content (*19*, *p.* 15).

(*43*), that lime-induced chlorosis is caused chiefly by a lack of potash in the plant ash. This opinion is evidently based only on the analyses of Fliche and Grandeau (*9*, *10*, *11*) and on those of Schulze (*42*). If a lack of potash in the ash were the cause of the chlorosis, plants grown in non-calcareous soils and in water cultures, under controlled conditions, with an insufficient supply of potash, should show this type of chlorosis. In such cases, however, the lack of potash is indicated by the appearance of brown spots on the leaves and not by a yellowing.[1] However, it has not been shown that a combined excess of lime and deficiency of potash would not produce chlorosis.

The reliability of ash analyses as the sole means of diagnosing the cause of chlorosis is questionable. At the most, the results of ash analyses should be taken as merely indicating the cause or as confirming other evidence. The ash compositions of normal plants show such wide variations and are affected by so many conditions that it is sometimes unsafe to assume that of two lots of plants those which have made the better growth have an ash composition more nearly normal.

Aside from difficulties in properly interpreting the results of ash analyses, it is sometimes doubtful whether the samples selected for analysis are truly comparable, even when whole plants are taken. This uncertainty was demonstrated in the analyses of rice, previously referred to. The practice of taking only a portion of a plant for analysis is also susceptible to error, especially where iron is to be determined. Since iron appears to be relatively immobile in the plant after it is once transported to the leaves, certain leaves of a plant might contain a sufficiency of iron while other leaves and the plant as a whole might lack iron (*16*).

EFFECT OF APPLICATION OF IRON SALTS TO CHLOROTIC PLANTS

Eusebe Gris, in 1845 (*20*), and later Sachs (*41*) and other investigators (*12*, *21*, *25*, *26*, *27*) showed that various plants which became chlorotic on calcareous soils could be cured by applying ferrous sulphate to the leaves. This treatment and the improved one of Rassiguier (*37*), that of brushing cut surfaces of pruned vines with a concentrated solution of ferrous sulphate, have been rather generally used on grapevines which became chlorotic on the calcareous soils of France and Germany.

Various investigators have found that while iron salts were effective in overcoming chlorosis when applied to the stems and leaves of plants, they were ineffective when applied to the soil, even if used in considerable quantity. Sachs (*41*), however, observed that where the roots of plants were not completely surrounded by earth, as in the case of pot-bound plants, applications of ferrous sulphate to the soil did cure the chlorosis.

[1] If potash is concerned in the formation of starch from sugars, a low percentage of potash in chlorotic plants might be a secondary result of the chlorosis. With insufficient iron, chlorophyll formation is depressed, less sugar can be synthesized, and little potash would be needed.

Since ferrous sulphate is, of course, immediately transformed into ferric carbonate in a calcareous soil, it seems evident that calcium carbonate renders ferric carbonate unavailable, or less available, to certain plants.

It has been repeatedly demonstrated that the effectiveness of spraying with ferrous sulphate is due only to the iron and that only soluble iron salts are effective (*12*, *21*, *25*, *26*, *27*).

EXPERIMENT I.—The results in Table I show the effect of an iron spray upon chlorotic rice growing in a calcareous soil. The plants were grown in the open from February 29 to July 13, 1912, in small brick compartments with 36 plants to each compartment. Each compartment held about 200 pounds of heavy loam soil and received 5 gm. nitrogen, 3.4 gm. phosphoric acid, and 5 gm. potash, derived from various commercial fertilizers. The plants sprayed with ferrous sulphate were given 4 applications of a 0.5 per cent solution and 12 applications of a 1 per cent solution.

TABLE I.—*Effect of an iron spray upon chlorotic rice plants grown on calcareous soils*

Test No.	Calcium carbonate content of soil.	Treatment of plants.	Green weight of plants per compartment.		
			Series A.	Series B.	Average.
	Per cent.		*Gm.*	*Gm.*	*Gm.*
1	0	Unsprayed	1,022	1,071	1,047
2	0	Sprayed with ferrous sulphate	1,088	1,040	1,064
3	30	Unsprayed	4	(a)	.....
4	30	Sprayed with ferrous sulphate	946	894	920
5	50	Unsprayed	242	702	472
6	50	Sprayed with ferrous sulphate	874	893	884

a Some plants were eaten by mole cricket, but according to comparative growths of plants before any were eaten, the weight would have been about 250 gm.

Twenty-one days after planting, the plants in the calcareous soils were markedly chlorotic, and spraying was begun at that time. Seven days later, after nine sprayings, the sprayed plants in the calcareous soils were much superior to the unsprayed in color and growth. All plants in the noncalcareous soil had a good color at all times. (Pl. 5, A.)

The results obtained by treating the leaves and stems of chlorotic plants with iron salts show clearly that a lack of iron in the plant is at least one of the causes of lime-induced chlorosis. This conclusion is substantiated by the results of ash analyses of the plants. But this work does not show: (1) whether the lack of iron in the plant is due to a low availability of iron in the soil or to reactions in the plant rendering ineffective the iron absorbed; (2) whether an increased absorption of lime is a contributory cause of chlorosis; or (3) whether the reaction of the soil has any effect on the appearance of chlorosis, aside from affecting the iron supply.

EFFECT OF COMPOUNDS OF LIME IN INDUCING CHLOROSIS

To see whether lime salts in general induce chlorosis in certain plants, experiments have been conducted with calcium carbonate, sulphate, phosphate, and silicate. The effects of these compounds on the growth of lupines have been determined by Heinrich (*23*), Pfeiffer and Blanck (*35*), and Creydt (*5*). The calcium sulphate did not induce chlorosis but depressed growth considerably, although much less than the calcium carbonate, while calcium phosphate and silicate were markedly toxic. The toxicities of the latter two substances were attributed to their acid and alkaline reactions, respectively.

Large quantities of gypsum depressed the growth of pineapples about 20 per cent but did not cause chlorosis (*12*). Various experiments were conducted to determine the effect on rice of large amounts of assimilable lime in the form of gypsum.

EXPERIMENT II.—In this experiment, rice plants were grown from December 17, 1912, to May 20, 1913, in small brick compartments, with 24 plants to each compartment. Each compartment held about 200 pounds of soil fertilized with 30 gm. sulphate of ammonia, 20 gm. nitrate of soda, 30 gm. acid phosphate, and 18 gm. muriate of potash added in two applications. The results are shown in Table II.

TABLE II.—*Effect on the growth of rice of adding gypsum to the soil*

Test No.	Kind of soil	Gypsum ($CaSO_4 \cdot 2H_2O$) added.	Green weight of plants per compartment.			
			Series A.	Series B.	Series C.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Loam	0	1,218	1,229	1,452	1,300
2	.....do	15	312	446	382	380
3	Clay	0	.....	808	840	824
4	.....do	15	936	958	842	912

During the first four weeks the plants were all of good color, but later the plants in the loam soil containing gypsum became yellow, though not typically chlorotic.

EXPERIMENT III.—A second experiment was conducted to see whether large amounts of gypsum would depress the growth of rice if the plants were sprayed with ferrous sulphate. The compartments contained about 200 pounds of a sandy soil and received 45 gm. sulphate of ammonia, 30 gm. acid phosphate, and 18 gm. muriate of potash. In each compartment 22 plants were grown. The plants treated with ferrous sulphate were sprayed twice with a 0.1 per cent solution, five times with a 0.15 per cent solution, once with a 0.2 per cent solution, three times with a 0.75 per cent solution, and seven times with a 1 per cent solution. The results are given in Table III.

TABLE III.—*Influence of spraying with ferrous sulphate on the depressing effect of gypsum*

Test No.	Gypsum ($CaSO_4 \cdot 2H_2O$) added.	Treatment of plants.	Air-dried weight of plants per compartment.			
			Series A.	Series B.	Series C.	Average.
	Per cent.		*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0	Unsprayed.....	521	503	475	500
2	0	Sprayed with ferrous sulphate.	478	525	525	509
3	15	Unsprayed.....	324	300	366	330
4	15	Sprayed with ferrous sulphate.	395	364	372	377

The plants in the soil to which gypsum had been added were markedly behind the others in growth from the start, and they were at times of poorer color, though they were never typically chlorotic. Plants in soil without gypsum were of good color at all times. No effect from spraying with ferrous sulphate was observable.

EXPERIMENT IV.—A further experiment with gypsum and ferrous sulphate was conducted in pots in a glass house. Six rice plants per pot were grown from July 7 to October 25, 1913. Each pot contained 37 pounds of sandy soil, to which 13 gm. ammonium sulphate, 11 gm. acid potassium phosphate, and 3.6 gm. sulphate of potash were applied. The moisture content of the soil was maintained at the optimum. The results appear in Table IV.

TABLE IV.—*Influence of different treatments with ferrous sulphate on the depressing effect of gypsum*

Test No.	Gypsum ($CaSO_4 \cdot 2H_2O$) added.	Treatment of plants.	Air-dried weight of plants per pot.				
			Series A.	Series B.	Series C.	Series D.	Average.
	Per cent.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0	.....	114	113	113	118	115
2	15	.....	85	92	68	67	78
3	15	Ferrous sulphate, 2.2 gm., added to soil.	.....	50	86	85	74
4	15	Plants sprayed eight times with 1 per cent solution of ferrous sulphate.	67	60	60	78	66

The color of the plants grown in the soil to which gypsum was added was as good as that of the controls up to the eighty-fifth day, but from the eighty-fifth to the one hundred and tenth day the former were yellow. The controls were always of a good green color. No effect from either of the treatments with ferrous sulphate was observable.

SUMMARY.—In all the tests, except that with the clay soil, calcium sulphate depressed the growth of rice and induced a certain amount of yellowing. The yellowing, however, was not that typical of lime-induced

chlorosis. Spraying with ferrous sulphate and adding ferrous sulphate to the soil failed to increase the growth or improve the color of plants growing in the soil containing calcium sulphate. That calcium sulphate increased the amount of lime in the plants may be seen by the analyses in Table V of plants 65 days old from experiment II.

TABLE V.—*Ash analyses of plants from experiment II*

Test No.	Kind of soil.	Gypsum ($CaSO_4 \cdot 2H_2O$) added.	Carbon-free ash in dry substance of plants.	Analyses of carbon-free ash.							
				Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Potash (K_2O).	Soda (Na_2O).	Iron (Fe_2O_3).	Phosphoric acid (P_2O_5).	Sulphur (SO_3).
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	Loam..	0	16.75	54.70	3.63	4.20	24.77	5.48	0.55	2.58	2.19
2	...do...	15	13.92	47.24	6.45	5.22	26.81	4.54	.53	4.01	6.71
3	Clay...	0	14.14	50.77	3.87	4.80	25.11	4.65	.62	2.31	2.74
4	...do...	15	13.08	45.04	6.40	4.77	28.34	7.00	.38	3.13	4.76

It will be noted that the calcium sulphate increased the percentages of lime and sulphur in the plant ash and diminished the percentage of silicia but had little effect on the other constituents.

The injurious effect of calcium sulphate on rice might have been due to several causes. A large amount of gypsum evidently maintains a solution more concentrated than that existing in any except alkali soils. There is also the possibility of hydrogen sulphid being formed by bacteria reducing sulphates. This occurred with soil preserved in a sample jar, although such a result was not to be expected in what appeared to be a normally aerated soil. The fact that calcium sulphate did not depress growth in the clay soil lends credence to the view that the injurious effect might have been that of a too concentrated soil solution.

In order to make sure that an increased assimilation of lime is not a cause of chlorosis, a test was conducted with lime salts applied to the leaves. The results, given in experiments V and VI, to be described further on, seemed to show definitely that an increased assimilation of lime does not induce chlorosis.

Although excessive quantities of various lime compounds seem to be more or less injurious, each one appears to act differently; there is no evidence of a general "lime effect" in inducing chlorosis.

EFFECT OF AN ALKALINE REACTION IN INDUCING CHLOROSIS

Pfeiffer and Blanck (*35*) in their first work on the intolerance of lupines for calcareous soils concluded that lupines are especially sensitive to an alkaline reaction and that the carbonate of lime not only depresses the absorption of nutrients but is directly injurious to the roots of the plants. While the alkaline reaction of carbonate of lime is evidently a factor in the chlorosis, it is very evidently not directly injurious to roots of even calcifugous plants. It was found in experiments with pineapple and

rice at this Station that the ratio of root to top growth was much increased in calcareous soils and solutions (*12*, *17*). The stimulating effect of carbonate of lime on the root growth of plants which are not calcifugous has been frequently noted.

In work with pineapples it was shown that the alkalinity induced by increasing amounts of carbonate of soda greatly depressed growth without affecting the color of the plants (*12*, *p. 31*).

Work with rice in water cultures seemed to show definitely that the alkalinity of carbonate of lime is not directly injurious to this calcifugous plant, nor is the alkalinity in itself the cause of chlorosis (*17*). Rice was grown with different quantities of iron from different sources in nutrient solutions which were acid, neutral, and alkaline from carbonate of lime. A summary of the relative growths made under the different conditions is given in Table VI.

TABLE VI.—*Relative growths of rice plants with different amounts of iron from various sources in acid, neutral, and alkaline solutions*

Source of iron in nutrient solutions.	Iron per liter added to nutrient solutions.	Relative growths in—		
		Acid solution.	Neutral solution.	Alkaline solution.
	Gm.			
Ferrous sulphate	0.002	100	88	.....
Do	.....	100	74	51
Do	.....	100	95	95
Do	.008	100	105	.....
Do	.004			
Do	.002	100	107	.....
Do	.008	100	132	.....
Do	.....	100	111	2
Ferric chlorid	.002	100	99	26
Do	.008	100	107	26
Ferric citrate	.002	100	85	86
Do	.....	100	94	97
Do	.....	100	101	104
Do	.008	100	85	58
Ferric tartrate	.002	100	80	76
Do	.008	100	96	100
Dialyzed iron	.008	100	27	.....

Where growth was depressed to any extent the plants were more or less chlorotic, and that this chlorosis was evidently due to lack of iron was shown by analyses of the plants and by treatment of the leaves with ferrous sulphate. The work showed quite definitely that rice is not particularly sensitive to the reaction of carbonate of lime, except as the reaction influences the availability of the iron. When the proper form of iron was used in the proper quantity, the growth and appearance of the plants were as good in the solutions containing carbonate of lime as in the acid or neutral solutions.

The preceding results seem to show that neither increased assimilation of lime nor mere alkalinity causes chlorosis. It remained to be seen

whether the combination of the two conditions would induce a typical chlorosis. It was thought that this might be determined experimentally by growing rice on soil to which sodium bicarbonate had been added to render it alkaline and then inducing an increased assimilation of lime by spraying the plants with calcium chlorid and gypsum. In case these treatments should induce a chlorosis identical with that produced by carbonate of lime, spraying with ferrous sulphate should cure it. Accordingly, some plants grown in the soil with sodium bicarbonate were sprayed with lime salts alone, with ferrous sulphate alone, and with both lime and iron salts. Plants grown in a soil without sodium bicarbonate were also sprayed as described above in order to check the results.

The experiment was carried out twice, once in the open, using small brick compartments, and once in the glass house, using pots. Results are given under the heads of experiments V and VI. The sodium bicarbonate was added in several doses until it became evident that sufficient had been applied to affect growth. More was required for the soil in the open than for that in the glass house, since the former was exposed to leaching.

EXPERIMENT V.—This test was run from November 8, 1913, to January 20, 1914. Each plot containing 150 pounds of sandy soil received 45 gm. sulphate of ammonia, 30 gm. acid phosphate, and 18 gm. muriate of potash. Thirty rice plants were grown on each plot. The results are given in Table VII.

TABLE VII.—*Effect of sodium bicarbonate, lime, and iron on the growth of rice: Experiment V*

Test No.	Approximate percentage of sodium bicarbonate in soil.	Treatment.	Air-dried weight of plants per plot.		
			Series A.	Series B.	Average.
			Gm.	*Gm.*	*Gm.*
1	0	None	144	156	150
2	0	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum	142	152	147
3	0	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum and 8 times with 0.5 to 1 per cent solutions of ferrous sulphate	163	166	165
4	0.2	None	100	104	102
5	.2	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum	101	82	92
6	.2	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum and 8 times with 0.5 to 1 per cent solutions of ferrous sulphate	110	100	105
7	.2	Sprayed 8 times with 0.5 to 1 per cent solutions of ferrous sulphate	.....	113	113

The plants in the soils containing sodium bicarbonate became somewhat yellow, though the yellowing was not that of typical lime-induced chlorosis. The yellowing, however, was not increased by the lime spray, nor was it overcome by the iron spray. The lime and iron sprays also had no effect on the appearance of the plants growing in the soil containing no sodium bicarbonate.

EXPERIMENT VI.—In this test, conducted from November 4, 1913, to March 12, 1914, 7 rice plants were grown per pot. Each pot contained 35 pounds of sandy soil and received 6 gm. ammonium nitrate, 1.3 gm. potassium acid phosphate, and 2.5 gm. potassium sulphate. The moisture content was maintained at 25 per cent of the dry weight of the soil. The results are shown in Table VIII.

TABLE VIII.—*Effect of sodium bicarbonate, lime, and iron on the growth of rice: Experiment VI*

Test No.	Approximate percentage of sodium bicarbonate in soil.	Treatment.	Air-dried weight of plants per pot.		
			Series A.	Series B.	Average.
1	0	None	*Gm.*	*Gm.*	*Gm.*
2	0	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	83	71	77
3	0	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 7 times with 0.5 to 1 per cent solutions of ferrous sulphate	68	72	70
4	0.08	None	65	70	68
5	.08	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	44	56	50
6	.08	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 7 times with 0.5 to 1 per cent solutions of ferrous sulphate	52	51	50
7	.08	Plants sprayed 7 times with 0.5 to 1 per cent solutions of ferrous sulphate	42	39	40
			49	60	55

The appearance of the plants in this test was the same as in experiment V.

The plants from experiment V were analyzed for their ash constituents, and the results appear in Table IX. The plants were washed immediately after cutting, so no lime salts remained on the leaves. While it is believed that all iron applied as a spray was also removed by washing, it is possible that some iron in the form of ferric oxid might have remained adhering to the leaves; hence, in the case of the plants sprayed with ferrous sulphate, it is possible that the analytical results may show more iron than was actually present in the plants.

TABLE IX.—*Analyses of rice plants growing in a soil with sodium bicarbonate and sprayed with lime and iron salts*

CONSTITUENTS OF ASH IN TOTAL DRY MATTER

Test No.	Approximate percentage of sodium bicarbonate in the soil.	Treatment.	Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Potash (K_2O).	Soda (Na_2O).	Iron (Fe_2O_3).	Phosphoric acid (P_2O_5).	Nitrogen (N).
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	0	None	11.17	4.51	0.48	0.59	3.75	0.30	0.015	0.72	2.57
2	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	14.12	6.83	.83	.64	4.31	.35	.021	.62	2.57
3	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	12.80	5.88	.71	.39	3.30	.47	.065	.69	2.74
4	0.2	None	14.98	8.06	.54	.82	3.44	1.02	.018	.69	2.80
5	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	15.56	7.84	1.10	.70	3.44	.88	.019	.66	2.91
6	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	14.96	7.78	.81	.66	3.43	.73	.084	.68	2.73
7	.2	Plants sprayed with 0.5 to 1 per cent solutions of ferrous sulphate	12.41	5.98	.51	.68	3.20	.91	.063	.64	.....

CONSTITUENTS OF CARBON-FREE ASH

1	0	None	.....	40.42	4.29	5.27	33.61	2.72	0.14	6.44	.....
2	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	.....	48.46	5.87	4.50	30.48	2.45	.15	4.39	.....
3	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	.....	46.01	5.53	3.04	29.72	3.70	.51	5.41	.....
4	0.2	None	.....	53.90	3.60	5.46	22.95	6.80	.12	4.59	.....
5	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	.....	50.34	7.07	4.52	22.18	5.65	.12	4.26	.....
6	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	.....	52.01	5.74	4.44	22.96	4.91	.56	4.54	.....
7	.2	Plants sprayed with 0.5 to 1 per cent solutions of ferrous sulphate	.....	48.13	4.10	5.46	25.77	7.30	.51	5.18	.....

SUMMARY.—None of the sprays affected the growth or color of the plants, either in the normal soil or in the soil containing sodium bicarbonate. The amount of sodium bicarbonate required to depress growth was rather surprising, and from this fact it was suspected that the availability of iron was not noticeably depressed by sodium bicarbonate, at least not below the critical point. This was confirmed by the analyses of the plants and by the fact that spraying with ferrous sulphate effected no improvement in either the growth or color of the plants planted in the soil containing sodium bicarbonate.

The spraying with lime salts, however, notably increased the amount of lime in the plants without affecting the quantity of iron, and spraying with both lime and iron solutions increased the quantities of both elements in the plant. The yellowing and depression in growth produced by the sodium bicarbonate were probably due to an injurious degree of alkalinity, which must have been far greater than that which is produced by carbonate of lime.

The results of these experiments, where a large amount of sodium bicarbonate was required to depress growth, seem to show that the slight alkalinity of carbonate of lime could not be directly injurious to rice, nor could alkalinity in itself be the cause of chlorosis. While this experiment failed to yield the decisive answer expected, it is felt that the results point strongly to the conclusion that an increased assimilation of lime is not the cause of chlorosis.

CHLOROSIS DUE SIMPLY TO A DEPRESSION IN AVAILABILITY OF IRON IN THE SOIL

An attempt was made to demonstrate directly that the only action of carbonate of lime in inducing chlorosis lies in depressing the availability of the iron. It was thought that this demonstration could be accomplished by growing rice plants with their roots divided between two kinds of soil, one to contain carbonate of lime and all the mineral nutrients except iron, and the other to contain only iron. The attempt was not completely successful, due partly to a principle discovered later and partly to difficulties in execution. The principle which tended to make the results less striking than had been anticipated is the following: Plants apparently are unable to attain a maximum absorption of any one element with only a part of their roots (*18*).

Wire sieves were made which fitted into the tops of buckets. The buckets were filled with soil to within 1 inch of the bottom of the sieves, and the sieves were filled with about 2 inches of soil (Pl. 5, B). In this way an air space was left between the soil in the sieve and that in the bucket; this prevented any soil solution passing by capillary attraction from the soil below to that above. It was the intention at first to fill all except the control buckets with a calcareous soil containing all the nitrogen, phosphoric acid, and potash, and to fill most sieves with pure silica sand containing only iron. In conducting the experiment,

however, it was found necessary to apply a small amount of nitrogen, phosphoric acid, and potash to the sand in the sieve in order that the plants might develop sufficiently for their roots to penetrate the soil below.

The intention was that the plants should absorb practically all their nutrients from the soil in the buckets (a calcareous soil except in control buckets 1 to 4), but that in some cases the plants should be able to absorb iron from a lime-free medium in the sieve. If carbonate of lime affected the plants in any way except through depressing the absorption of iron, all plants should make equally poor growth; but if, on the other hand, the only action of the carbonate of lime lay in decreasing the availability of the iron, those plants that could draw iron from a medium containing no carbonate of lime should do much better than the others.

A preliminary test was run with two pots, No. 1 containing silica sand in the sieve and a calcareous soil in the bucket, and No. 2 containing silica sand plus carbonate of lime in the sieve and the same calcareous soil in the bucket as No. 1. Four gm. of ferrous sulphate were applied to both sieves (Pl. 6, A). The yields from pots No. 1 and 2 were respectively 169 gm. and 97 gm. of air-dried plants, the plants in No. 1 being green in color and those in No. 2 chlorotic.

EXPERIMENT VII.—The results of a more extended test are given in Table X. The plants were grown from October 22, 1912, to March 3, 1913. A large number of seeds were planted, but the plants in each pot were thinned to eight. The sieve of each pot contained 10 pounds of silica sand to which were added 0.45 gm. ammonium nitrate, 0.1 gm. acid potassium phosphate, and 0.2 gm. potassium sulphate. The bucket of each pot contained 23 pounds of soil and received 12 gm. ammonium nitrate, 3 gm. acid potassium phosphate, and 5.5 gm. potassium sulphate, in two applications. The moisture content of the soil was maintained at 31 per cent of the dry weight.

TABLE X.—*Effect of carbonate of lime in the soil on the availability of iron*

Pot No.	Treatment of soil in bucket.	Treatment of sand in sieve.	Green weight of plants per pot.				
			Series A.	Series B.	Series C.	Series D.	Average.
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1 to 4...	None.....	None.....	206	171	204	.....	194
5 to 8...	Calcium carbonate, 15 per cent.	None.....	137	224	156	161	170
9 to 12..	.....do.....	Eight gm. ferrous sulphate in four applications.	204	174	236	187	200
13 to 16.	.....do.....	Eight gm. ferrous sulphate in four applications; 15 per cent calcium carbonate.	112	97	115	84	102

At 15 days after sowing the seed all plants were chlorotic except those in pots 13 to 16, and many died because of their inability to establish roots in the soil in the bucket. At 121 days the plants of pots 1 to 4 and 9 to 12 were green, while those of No. 5 to 8 and 13 to 16 were strongly chlorotic.

The plants encountered some difficulty in establishing their roots in the soil in the buckets; the roots after passing through the sieve often grew for a time on the surface of the soil. This retarded growth considerably, but when the roots once penetrated the soil, growth became normal. At the end of the experiment the greater part of the roots were in the soil in the bucket, where practically all the fertilizer was located.

The final yields of the plants and the chlorotic appearance of certain plants during the latter stages of growth confirm the idea that the only effect of carbonate of lime in inducing chlorosis lies in depressing the availability of iron. The plants in pots No. 9 to 12 and those in No. 13 to 16 were exposed to the same conditions except that the plants in No. 9 to 12 were able to draw part of their iron from a medium containing no carbonate of lime; this difference was sufficient to double the growth of plants. The plants of No. 9 to 12 had to assimilate practically all their mineral nutrients, except iron, from the same calcareous soil as the plants of No. 13 to 16; hence, if the carbonate of lime induced chlorosis by depressing the availability of any nutrients other than iron, or if an increased assimilation of lime were a contributory cause of chlorosis, the yield from pots No. 9 to 12 should have been practically the same as from No. 13 to 16.

The only apparent contradiction in this demonstration of the cause of lime-induced chlorosis lies in the fact pots No. 5 to 8 yielded more than No. 13 to 16. Plants in pots No. 5 to 8 evidently secured less iron than those in No. 9 to 12, for they made less growth; but if the sand in the sieve had been really iron-free they should have made no more growth than plants No. 13 to 16. Later work showed that, although no iron was added to the sieves of No. 5 to 8, doubtless the silica sand contained enough iron to cause the unanticipated growth. In work with nutrient solutions it was found that rice practically satisfied its iron requirements in a solution containing no more than 1 part of truly soluble iron in 10,000,000 parts of solution (*17, p. 5*).

On repeating this experiment the same difficulties were encountered, but the relative growths made by the differently treated plants were similar to those in the preceding test.

AVAILABILITY OF IRON IN THE SOIL

INTRODUCTION

Since the preceding summary of facts and experiments seems to indicate that lime-induced chlorosis is simply the result of insufficient available iron in the soil, evidently a knowledge of conditions affecting the

availabiiity of iron in the soil is essential to a complete understanding of this chlorosis. If all the conditions affecting the amount of available iron in the soil were known, it would doubtless be possible to explain why some calcareous soils induce chlorosis when others do not; why in a sandy soil a smaller percentage of carbonate of lime is required to induce chlorosis than in a clay soil; why a calcareous soil that produces chlorotic plants at one time may not at another; and many other perplexing facts.

Since a method for determining the amount of available potash or phosphoric acid in the soil is still unknown, in spite of years of work, the prospect is not bright for even roughly determining the available iron by direct means; and to determine directly significant differences in amounts of available iron seems hopeless when plants obtain their iron from such exceedingly dilute solutions.

Soils which yield sufficient iron for the growth of plants may not show a detectable amount of iron in the water extract. In some cases the water extract of soils may show considerable iron, but the iron may be in a colloidal state and not in true solution. Colloidal iron was found unavailable for rice in water culture (*14*).

While there are great difficulties in the way of determining the small, significant quantities of soluble or available iron in the soil, it seems from the work of Morse and Curry (*34*), Ruprecht (*40*), and Abbott (*1*) that acid soils may contain much more soluble iron and aluminum than neutral or calcareous soils and may even contain an injurious amount of these compounds.

The following work on the availability of iron compounds is based on the assumption that the chlorosis and the poor growth of rice in the calcareous soils were caused by a lack of available iron. This assumption seems justified by the results presented in the first part of this report.

AVAILABILITY OF ORGANIC IRON COMPOUNDS

In work with pineapples it developed that in the presence of a great amount of organic matter a large amount of carbonate of lime was required to induce chlorosis (*12*). This suggested that in calcareous soils organic iron compounds might be more available than the inorganic, just as iron in solution as a complex ion is less completely precipitated by the usual reagents. The idea seemed substantiated by tests with rice in nutrient solutions containing carbonate of lime, where ferric tartrate furnished much more available iron than equivalent quantities of ferrous sulphate or ferric chlorid.

EXPERIMENT VIII.—Tests were accordingly conducted to determine the effect of various iron compounds and organic materials on the growth of rice in both calcareous and noncalcareous soils. In this experiment the effects of certain pure organic compounds of iron were compared with those of ferric chlorid and ferrous sulphate. A substance which

may be called "ferric molasses" was also used. This was prepared by boiling together 2 parts of ferrous sulphate and 10 parts of a final molasses. It doubtless contained some ferric acetate, glucolate, laevulate, possibly other organic iron compounds, and considerable inorganic iron. As a control on the action of the "ferric molasses," the same quantity of molasses which had been similarly boiled without addition of iron was applied to two other lots of pots. To one of these lots ferrous sulphate was applied after the boiled molasses had been mixed with the soil in the pots designated as "molasses and ferrous sulphate" in Table XI.

Five rice plants were grown in each pot from September 28 to December 28, 1914. In the noncalcareous series each pot contained 14 pounds of loamy soil with the moisture content maintained at 23 per cent of the dry weight; and in the calcareous series each pot contained 14 pounds of loamy soil with the moisture content maintained at 27 per cent of the dry weight. The calcareous soil contained 17.8 per cent of carbonate of lime. A fertilizer consisting of 1.8 gm. ammonium nitrate, 4.2 gm. sodium nitrate, 3 gm. ammonium sulphate, 0.4 gm. acid potassium phosphate, 3.9 gm. acid phosphate, and 3.8 gm. potassium sulphate was added to each pot in four applications. The molasses and all the iron compounds were mixed with the soil before the rice was planted. The iron was applied at the rate of 0.25 gm. and the molasses at the rate of 6.25 gm. per pot. The results of the experiment are summarized in Table XI.

TABLE XI.—*Comparative availability to rice plants of organic and inorganic compounds of iron in a calcareous and noncalcareous soil: Experiment VIII*

Special additions to the soil.	Oven-dried yield of plants per pot.											
	Calcareous soil.						Noncalcareous soil.					
	Series A.	Series B.	Series C.	Series D.	Series E.	Average.	Series A.	Series B.	Series C.	Series D.	Series E.	Average.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm*
.....	20	16	9	12	13	14	39	41	38	41	59	44
Ferric chlorid.....	16	18	7	8	15	13	.....	.....	.....	.....	.....	.....
Ferric tartrate.....	12	21	26	12	26	19	49	55	57	43	50	51
Ferric citrate.....	18	12	13	23	7	15	51	42	43	42	46	45
Ferric valerianate.....	11	18	8	7	10	11	49	58	51	44	48	50
Ferric benzoate.....	9	20	34	18	18	20	49	58	46	52	59	53
Molasses.....	7	5	2	2	3	4	52	54	41	50	54	50
"Ferric molasses".....	18	7	22	15	14	15	42	44	55	50	60	50
Molasses and ferrous sulphate.....	6	9	4	5	4	6	40	49	54	49	56	50
Ferrous sulphate.....	16	13	21	15	7	14	.....	.....	.....	.....	.....	.....

Three weeks after planting, all plants in the noncalcareous soil were green, while many plants in the calcareous soil were slightly chlorotic. Those plants in the calcareous soil which received molasses alone or molasses with ferrous sulphate were markedly chlorotic (Pl. 6, B). During later growth the plants in noncalcareous soil remained green and those in calcareous soil became more chlorotic, some plants eventually dying from the top down.

In the noncalcareous soil none of the special compounds affected growth significantly, and in the calcareous soil none of the iron compounds proved efficient sources of iron, although possibly the ferric tartrate and benzoate increased growth slightly.

Molasses alone and molasses followed by ferrous sulphate depressed growth markedly and intensified the chlorosis of plants in the calcareous soil, but the "ferric molasses" had no effect. Probably the molasses that had not been treated with iron still further depressed the availability of iron in the calcareous soils by promoting the formation of insoluble organic iron compounds.

EXPERIMENT IX.—Later a second test was conducted with pure organic iron compounds and organic materials containing iron in calcareous and noncalcareous soils. The pure iron compounds were applied so as to furnish 0.75 gm. or 1.50 gm. of iron per pot, the smaller application being at approximately the same rate as in the preceding experiment, if the sizes of the pots and quantities of soil used in the two experiments are considered. In the tests with ferric citrate and ferric tartrate, a comparison was made between the results obtained by mixing all the material with the soil before planting and those obtained by applying the material in small doses in solution during the growth of the plants. This was done to see if the materials might not be available for a short time in the soil although rendered unavailable in the course of time by bacterial or other action.

The "ferric humate," which, it was thought, might contain some iron compounds similar to those existing in a natural soil, was prepared by extracting leaf mold with 4 per cent ammonia, acidifying with hydrochloric acid, washing the precipitate free from chlorids, and evaporating the precipitate to dryness with sufficient ferric chlorid solution to furnish 25 per cent as much iron as dry matter. The "mixture" used per pot was composed of 4 gm. dried blood, 40 gm. Stizolobium vines, 40 gm. tobacco stems, and 0.90 gm. iron from equal parts of ferric citrate, tartrate, "humate," tannate, oxalate, and benzoate. Velvet beans (Stizolobium) were tested because they are extensively grown as a green manure crop. Both Stizolobium vines and tobacco stems were cut up before mixing with the soil. Citric and tartaric acids were tried to see whether an organic radical alone would have any effect in maintaining available iron in the soil. The test was conducted from December 8, 1916, to February 10, 1917, with eight rice plants in each pot. The pots contained 42 pounds of sandy loam soil, or 47 pounds of sandy soil containing 10 per cent carbonate of lime. The moisture contents of both soils were maintained at 18 per cent of the dry weight. The fertilizer for each pot was given in two applications and consisted of 1.5 gm. ammonium sulphate, 10.5 gm. acid phosphate, and 6 gm. potassium sulphate. The special additions were mixed with the top 4 inches of soil before the rice was planted, except the solutions of ferric citrate

and ferric tartrate which were applied to the soil every other day. Results of the test are given in Table XII.

TABLE XII.—*Comparative availability to rice plants of organic and inorganic iron compounds in calcareous and noncalcareous soils: Experiment I*

Special additions to the soil.	Amount added.	Oven-dried yield of plants per pot.											
		Calcareous soil.						Noncalcareous soil					
		Series A.	Series B.	Series C.	Series D.	Series E.	Average.	Series A.	Series B.	Series C.	Series D.	Series E.	Average.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
None		28	22	23	26	26		60	67	68	65	75	
None		30	24	17	24	28	25	69	72	70	69	76	69
Ferric oxalate	2.43	18	21	26	24	24	21	72	70	66	56	75	69
Do	4.86	19	20	25	19	24	21	62	66	69	68	65	66
Ferric tannate	8.18	19	22	22	29	25	23	72	68	68	73	69	70
Do	16.36	23	28	23	22	28	25	69	68	77	68	75	71
"Ferric humate"	3.75	18	16	18	18	16	17	62	69	71	63	65	66
Do	7.50	20	16	13	16	16	16	59	61	66	63	56	61
Ferric citrate	8	24	21	24	22	24	23	63	64	65	71	64	65
Solution of ferric citrate		24	20	25	28	26	25	75	75	79	77	69	75
Ferric tartrate	9.34	27	20	22	24	21	23	71	69	74	66	62	68
Solution of ferric tartrate		23	26	15	25	26	23	67	78	72	75	57	70
Tobacco stems	40	28	30	26	33	31	30	62	64	66	72	67	66
Do	80	36	32	32	37	32	34	57	56	58	64	63	60
Stizolobium vines	40	24	26	24	32	25	26	74	60	62	66	70	68
Do	80	38	34	37	32	34	35	67	65	66	67	68	67
Dried blood	4	21	22	19	21	24	21	65	65	69	78	76	71
"Mixture"	92	28	27	26	30	26	27	59	54	57	59	56	57
Citric acid	10	29	23	32	32	23	28	72	68	66	58	77	68
Tartaric acid	10	28	33	26	36	28	30	71	67	71	68	73	70

After three weeks the plants in noncalcareous soil were about twice the size of those in calcareous soil. Later the plants in calcareous soil were all more or less chlorotic, but the plants in pots receiving the larger applications of tobacco stems, cover crop, or "mixture" were less chlorotic than others. All the plants in the noncalcareous soil were a good green throughout growth.

In the noncalcareous soil none of the materials significantly affected growth except the "mixture," which depressed the yield about 20 per cent. In the calcareous soil the "ferric humate" was distinctly injurious, while the larger applications of tobacco stems and Stizolobium vines were plainly beneficial, although they did not induce a normal growth.

SUMMARY.—All organic iron compounds tried in the two preceding experiments failed to increase appreciably the growth of rice in the calcareous soils. It is, therefore, probable that organic iron is no more available than inorganic iron in such soils.

While concentrated or soluble organic materials, such as dried blood, citric and tartaric acids, molasses, and a humus extract, failed to ameliorate the chlorosis, bulky organic materials, such as tobacco stems and velvet bean plants, when used in considerable quantities measurably improved the growth and color of the plants. Also, in previous work with pineapples and sugar cane large amounts of stable manure ameliorated

or completely overcame the chlorosis, although small amounts were without appreciable effect.

In view of the nonavailability of the concentrated organic iron compounds, it seems probable that the beneficial effect of the bulky organic materials was not due primarily to the addition of certain iron compounds that were available in the calcareous soil as a whole. It is more probable that the particles of organic material formed isolated centers or points where iron was more available than in the rest of the soil. The plants were not able to secure all the iron they needed from these points for the reason that plants are apparently not able to absorb a maximum amount of iron with only a portion of their roots (*18*).

It may seem that the results of the last two tests negative the conclusions arrived at in the experiments with rice grown in solutions containing carbonate of lime where organic iron compounds supplied sufficient available iron. Conditions in the nutrient solutions, however, were somewhat different from those in the soil. To begin with, in the nutrient solutions the plants obtained their iron from an ordinary solution that was more or less sterile and that was frequently renewed. In the soil, on the other hand, the plants probably obtained their nutrients from aqueous films surrounding the soil particles, and there is evidence that in films reactions may occur which do not take place in ordinary solutions. Furthermore, bacterial action in the soil might have destroyed rapidly certain of the organic compounds supplied.[1]

EFFECT OF WATER CONTENT OF SOIL ON THE AVAILABILITY OF IRON

At present we know little of the true soil solution or film moisture. It is evident, however, that the nature of the soil particles must influence the composition of the solution or substances dissolved in the enveloping film. In the films surrounding particles of calcium carbonate the amount of iron in solution must be greatly reduced, since the iron would be precipitated as ferric oxid.

If it is assumed that each particle in the soil is isolated and that the moisture films surrounding the individual particles are discontinuous, it would follow that the larger the proportion of particles which were carbonate of lime the less soluble iron there would be in the whole medium.

This assumption would explain why carbonate of lime is more effective in inducing chlorosis the more finely divided it is and why a certain quantity of carbonate of lime exerts a stronger influence in a sandy soil containing relatively few particles than in a clay soil containing a large number of particles.

However, the case is not so simple as is assumed above. The moisture films are not discontinuous but more or less continuous, the continuity

[1] The fact that ferric citrate and ferric tartrate were no more effective when applied in frequent small doses than when applied all at once is some evidence against the idea that the organic iron compounds were unavailable because they were destroyed by bacterial action.

and thickness of the films depending somewhat on the amount of moisture in the soil. The substances in solution in a film surrounding one particle will therefore react with those in films surrounding adjacent films. One particle of carbonate of lime would affect the soluble iron in the films of a certain number of adjacent particles.

While the moisture films are to a certain extent continuous, we know that the composition of the films is not uniform throughout the soil. This is evident from certain well-established facts, such as the slight lateral movement of fertilizers. If the composition of the films were uniform and conditions were analogous to those in a solution with relatively few solid particles, a slight amount of carbonate of lime would have the same effect as a much larger amount. This, however, is not the case.

It might be expected that the effect of carbonate of lime in depressing the availability of iron and in inducing chlorosis would be influenced somewhat by the amount of water in the soil, since the aggregation of the soil particles and their moisture films would be affected by the water content. It was, therefore, of interest to observe the manner in which the growth and chlorosis of rice would be affected by different percentages of moisture in calcareous soil.

A preliminary test was conducted with four pots, each of which held 36 pounds of soil containing 15 per cent of calcium carbonate. Twelve rice plants were grown in each pot with abundant fertilizer. The plants were grown 30 days with 22 per cent of moisture in the soil. Water was then added to two of the pots until there were 2 inches of water above the surface of the soil, and the other two pots were maintained unchanged at 22 per cent moisture. After 67 days' growth the plants were cut.

The plants in all four pots were very slightly chlorotic at 30 days, but a few days after the extra water was added the submerged plants became intensely chlorotic and remained so for about 10 days. They then quickly improved in color, and a few days later the submerged plants were a perfectly normal green, while the plants in the soil with 22 per cent moisture were markedly chlorotic. This difference persisted until the plants were cut. The plants grown for the whole period with 22 per cent moisture gave an average green weight of 175 gm. per pot, while the plants grown for 30 days with 22 per cent moisture and then submerged for 37 days yielded 424 gm. per pot.

EXPERIMENT X.—An extended test was conducted from January 2 to March 22, 1918, using one noncalcareous soil and two calcareous soils (one a beach sand with practically no organic matter and the other a loam).[1] The noncalcareous soil was used as a control to determine how the growth of rice would be affected by different amounts of water in a

[1] The calcareous loam was the same as the noncalcareous soil except for the addition of the carbonate of lime some years before.

soil adapted to its growth. Each pot received 9 gm. sulphate of potash, 6 gm. double superphosphate, and 22.5 gm. sulphate of ammonia divided in two applications. Twenty rice plants were planted in each pot, but these were thinned to 10 when growth was well established. The results are given in Table XIII.

TABLE XIII.—*Effect of varying degrees of moisture on the availability of iron to rice plants in calcareous and noncalcareous soils*

Soil No.	Kind of soil.	Percentage of calcium carbonate.	Optimum water content of soil expressed as percentage of dry weight of soil.	Maximum water capacity of soil expressed as percentage of dry weight of soil.	Amount of soil per pot.	Amount of water maintained in soil during growth of plant.	Oven-dried yield of plants per pot.			
							Series A.	Series B.	Series C.	Average.
					Pounds.		*Gm.*	*Gm.*	*Gm.*	*Gm.*
						22.3 per cent	119.8	122.1	103.9	115.3
						26.3 per cent	125.4	120.8	127.0	124.4
						30.3 per cent	146.1	128.9	137.7	137.6
1647	Loam	.....	25.5	34.3	69	34.3 per cent	129.2	142.1	141.6	137.6
						Water at surface of soil	159.9	167.5	153.8	160.4
						Water 3 inches above surface of soil	155.9	157.0	177.6	163.8
						20.2 per cent	58.1	51.5	57.5	55.7
						24.2 per cent	74.9	68.9	79.1	74.3
						28.2 per cent	53.7	70.8	78.8	67.8
1648	...do...	8.53	23.2	36.2	69	32.2 per cent	66.1	74.2	67.1	69.1
						36.2 per cent	87.3	72.9	77.8	79.3
						Water 3 inches above surface of soil	112.5	134.6	122.8	123.3
						11 per cent	12.4	9.9	9.2	10.5
						18 per cent	6.1	13.4	13.6	11.1
1194	Sand	19.0	11.6	25.0	98	25 per cent	1.2	9.4	1.4	4.0
						Water 3 inches above surface of soil	17.6	8.4	28.1	18.1

The different water contents maintained during the experiment were made up when the plants were 4 days old, except that the pots to receive 3 inches excess water were made up with water at the surface at this time, the water being raised to 3 inches when growth permitted it. When 11 days old, the plants in soils No. 1647 and 1648, where water was at the surface or above it, were markedly chlorotic, as well as all the plants in soil No. 1194. After 31 days' growth, all the plants in soil No. 1194 were still markedly chlorotic; the submerged plants in soil No. 1647 were normal green and were growing rapidly, as were all other plants in this soil; in soil No. 1648 the submerged plants and those in pots with 20.2 and 24.2 per cent water were normal green, while those in pots with 28.2, 32.2, and 36.2 per cent water were plainly chlorotic. At 72 days' growth, when the plants were cut, the appearance in regard to chlorosis was similar to that at 31 days, except that in soil No. 1194 the few plants that had not died in the pots with 3 inches excess water were normal green and far larger than the others.

The temporary chlorosis affecting the plants where the excess water was added is entirely distinct from the lime-induced chlorosis. A similar yellowing takes place in the field when the fields are flooded following

early growth without submergence. Several of the surplus plants in the pots with excess water were brushed repeatedly with ferrous sulphate, but the treatment did not improve the color of the plants in the slightest. Evidently this particular chlorosis is not due to lack of iron. Doubtless when the water content of the soil is raised above the point of saturation the old roots are unable to function properly and the nutrition of the plant is disturbed until new roots are sent forth which are able to function under the new conditions.

It was thought that roots of the submerged plants might show morphological differences from roots of plants grown with ordinary amounts of water in the soil. Samples of roots from plants grown in soil No. 1647 were therefore subjected to a preliminary examination by Dr. Albert Mann, of the Bureau of Plant Industry, United States Department of Agriculture, to whom thanks are due. A portion of Dr. Mann's report of the preliminary examination follows:

> The differences noted between No. 1805 with 24.2 per cent moisture, 1807 with 32.2 per cent, and 1809 with water standing three inches above the surface are slight. There is in general more compactness and strength of tissue in 1805 than in the others. The central fibrovascular bundle mass is larger in proportion to the cortex than in 1807 or 1809. The cells of all the tissues are slightly more robust. The light parenchyma, which makes up the cortex from the endodermal ring to the epiderm, is especially thinner walled and more developed in 1809. There is also a notable absence of root hairs in this sample as compared with the other two, which is, of course, the inevitable result of the roots growing submerged in water.

The series in the noncalcareous soil shows that the growth of rice should increase regularly with increasing amounts of water in the soil until a percentage near the saturation point of the soil is reached and that, possibly because of a different root growth, there should be another considerable increase when enough water is added for submergence. In No. 1648, however, the series with the calcareous soil, there were two maxima of growth, one at 24.2 per cent water and one at 3 inches excess; and in the calcareous sand No. 1194 there were also two maxima. It is believed that the first lower maximum was due to iron being a little more available at that water content than at a higher content. The great increase in growth in the calcareous soils produced by submergence [1] was probably due chiefly to the fact that the modified roots are better able to assimilate iron than the ordinary type of root and was probably not due to increased availability of iron in the submerged soil.

It is felt that the results substantiate the idea that the availability of iron in the soil is affected somewhat by the amount of water in the soil, the availability being slightly greater near the optimum water content than with larger amounts.

The effect of the water content is probably due to its influence on the extent to which reactions take place between the moisture films

[1] It will be noted that in the calcareous soils the increase produced by submergence was much greater than in the noncalcareous soil.

surrounding the calcareous particles and those surrounding the other soil particles. With moisture contents above the optimum the moisture films become more continuous and the sphere of influence of the particles of carbonate of lime in reducing the availability of iron becomes more extended.

Incidentally the tests established a fact of considerable practical importance—namely, that rice may be expected to make a practically normal growth in certain calcareous soils if the soils are submerged.

SUMMARY

There are a few plants which are generally conceded to be calcifugous, inasmuch as they are rarely found on calcareous soils.

Soil surveys of several species of cultivated plants show that a particular type of chlorosis affecting these plants occurs only on calcareous soils. All calcareous soils, however, do not induce chlorosis in these plants.

Addition of carbonate of lime to soils producing normal, calcifugous plants causes the soils to produce chlorotic plants.

It is, therefore, evident that a chlorosis of some plants is caused by, or is associated with, the presence of carbonate of lime in the soil.

The weight of the evidence from ash analyses of chlorotic plants seems to point to a deficiency of iron in the ash as being one cause of the chlorosis, with possibly an excess of lime as a contributory cause.

Treatment of chlorotic plants with iron shows that a lack of iron in the plant is at least one of the causes of lime-induced chlorosis.

There is no evidence of a general "lime effect" in inducing chlorosis, the different lime compounds affecting the plants differently.

Rice, one of the plants sensitive to lime, does not appear to be sensitive to the alkalinity of carbonate of lime except as this alkalinity influences the availability of the iron.

Lime-induced chlorosis seems to be due simply to a depression in the availability of iron in calcareous soils.

A number of pure organic iron compounds and concentrated organic preparations proved to be inefficient sources of iron for rice in calcareous soils. Bulky organic compounds such as stable manure, velvet bean plants, and tobacco stems, when used in considerable quantity, however, enabled the plant to secure more iron.

The availability of iron in calcareous soils appears to be slightly greater near the optimum water content of the soil than at higher percentages of water.

Although rice becomes chlorotic in calcareous soils with ordinary percentages of water, it will grow normally in certain calcareous soils if the soil is submerged. This is believed to be due to the growth, under submerged conditions, of a new kind of root that is better able to assimilate iron than the root formed in the soil with less water.

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PLATE 5

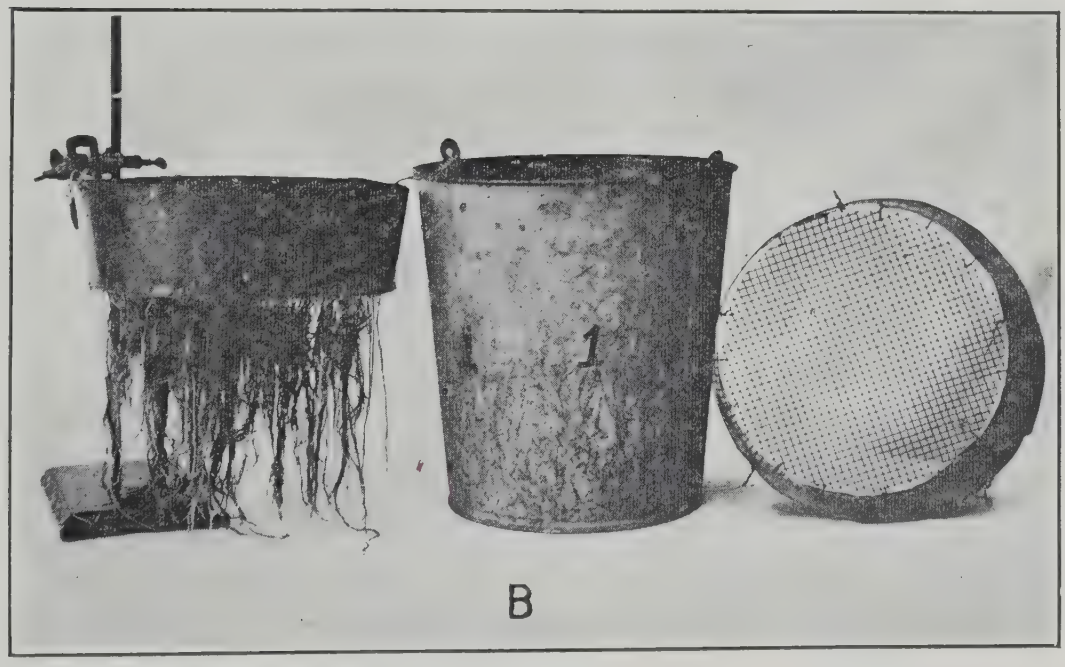
A.—Rice grown in calcareous and noncalcareous soils and sprayed with ferrous sulphate solution (experiment I).

1–4. Noncalcareous soil; plants in 1 and 3 unsprayed, those in 2 and 4 sprayed.

5–8. Soil containing 30 per cent carbonate of lime; plants in 5 and 7 unsprayed, those in 6 and 8 sprayed.

B.—Apparatus used in growing plants in experiment VII.


1
2
3
4
5
6
7
8
A


1
B


1
2
A


1
2
3
4
5
6
B

PLATE 6

A.—Effect of carbonate of lime in depressing the availability of iron (experiment VII).

1. Calcareous soil in bucket, silica sand plus iron in sieve.
2. Calcareous soil in bucket, silica sand plus carbonate of lime and iron in sieve.

B.—Effect of various substances on growth of rice in calcareous soil (experiment VIII).

1. Noncalcareous soil.
2. Calcareous soil.
3. Calcareous soil with molasses added.
4. Calcareous soil with "ferric molasses" added.
5. Calcareous soil with molasses and ferrous sulphate added.
6. Calcareous soil with ferrous sulphate added.

AN EXPERIMENTAL STUDY OF ECHINACEA THERAPY

By JAMES F. COUCH and LEIGH T. GILTNER, *Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The use of echinacea as a remedy for various disorders in both human beings and animals is said to have originated with the American Indians, from whom the early settlers in the West learned of the "virtues" of the plant.[1]

In 1885 Dr. H. C. F. Meyer, of Pawnee City, Nebr., sent a specimen of the plant to Prof. Lloyd. It was identified as *Echinacea angustifolia* (DC.). Dr. Meyer was using the root of this plant in a secret mixture which he called "Meyer's Blood Purifier," and the claims which he made for the curative properties of the root are described as "most exaggerated." Indeed, he had such confidence in it that he offered to submit to repeated bites of rattlesnakes, proposing to demonstrate the remedial power of echinacea against this venin by using his preparation of the root as an antidote. This offer was, of course, refused, but the drug was studied by Dr. John King, Prof. H. T. Webster, and others, with the result that clinical evidence was obtained which appeared to substantiate some of the claims of Dr. Meyer. Preparations of the plant were placed on the market, and the medicinal use of echinacea increased rapidly. Many physicians have reported favorable results from its use in various diseases.

In 1909 a report (*3*) of the Council on Chemistry and Pharmacy of the American Medical Association denied echinacea a place in "New and Non-Official Remedies" and directed suspicion at the value of the drug, stating:

> In view of the lack of any scientific scrutiny of the claims made for it, echinacea is deemed unworthy of further consideration until more reliable evidence is presented in its favor.

In spite of this the use of echinacea has become extensive. Lloyd states that it is used in larger quantities than any other American drug introduced since 1887. The fluid extract and tincture are made in enormous quantities, and the root enters into the composition of a large number of patent, proprietary, and nonsecret mixtures.

The last revision of the National Formulary includes a description of echinacea and furnishes a formula for its fluid extract (*1, p. 74, 294*). This amounts to a quasi official recognition of the drug. It has never been official in the United States Pharmacopœia.

[1] The historical facts about echinacea have been obtained almost wholly from published accounts by Meyer (*15*) and Lloyd (*11, 12, 13*). Reference is made by number (italic) to "Literature cited," p. 83–84.

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Five species of echinacea are mentioned in works on botany (*6*). *Brauneria purpurea* (DC.) (*Rudbeckia purpurea* or *Echinacea purpurea* [Moench]) is found from western Pennsylvania and Virginia to Michigan, Iowa, and southward. *B. angustifolia* (DC.) (*E. angustifolia* [DC.]) is found from Tennessee and Minnesota to Saskatchewan, Texas, and Nebraska. *B. pallida* (Nutt.) occurs from Michigan and Illinois to Texas and Alabama, while *B. paradoxa* (Norton) and *B. atrorubens* (Britton) (*R. pallida*) are found from Missouri to Texas. The rays of the last two species are bright yellow in color. The species which furnish the larger proportion of the medicinal supplies are *B. pallida* and *B. angustifolia*. It has been stated that the eastern species, *B. purpurea*, is inert.

CHEMICAL CONSTITUENTS

In 1897 Lloyd (*11*) reported the presence of a colorless alkaloid and a colorless acid-reacting substance of intensely acrid properties. The root has been subjected to analysis by Heyl and Staley (*8*) and Heyl and Hart (*7*), by whom the alkaloid was identified as betaine. Nothing of a physiologically active nature, however, was isolated by these investigators.

THERAPEUTIC USES

General accounts of the various uses to which echinacea has been put have been published by Ellingwood (*4*) and by Lloyd (*13*). Echinacea is stated to be a corrective of "depravation" of body fluids, of septic, fermentative, or zymotic conditions. It is said to antagonize infectious processes and "blood poison," to be useful in puerperal sepsis, uremia, pernicious malarial or septic fevers, typhoid fever, and all fevers caused by absorption of septic material. It has been recommended as a specific against the venins of rattlesnakes, other serpents, and insects (*9*)—

in crotalus it stands without a peer.

Pyemia, goiter, smallpox, anthrax, and hydrophobia are reported to have been cured by echinacea. It is said to be an antidote for tetanus. It has been used locally in erysipelas, bedsores, fever sores, chronic ulcers, glandular indurations, syphilitic nodules, burns, and gangrene (*14*) and is said to be an active sialogogue, diuretic, and diaphoretic. Jensen found it useful in the treatment of carbuncles.

The uses of echinacea in veterinary practice have been discussed by Fish (*5*), who found the root to increase the elimination of urea. In some pharmacological experiments upon kittens he obtained evidence of narcosis, and emesis was provoked by the fluid extract given per os. He quotes five cases in which the administration of echinacea was followed by improvement.

The compound of inula and echinacea prepared especially for parenteral administration has been stated to be useful in the treatment of tuberculosis (*18*), has been designated "an effective treatment for canine

distemper," and is recommended in the treatment of equine influenza (*10*). Slawson (*16*) does not consider this preparation satisfactory in the treatment of canine distemper. He finds that its action does not differ from that of nuclein, leucocyte extract, or plain serum.

PRESENT INVESTIGATION

The investigation of which the results are here reported was undertaken for the purpose of determining, so far as the limits of laboratory experiment permit, the usefulness of echinacea as a remedy in several pathological conditions induced by bacteria, their products, or allied toxins.

The animals used were guinea pigs bred at the Bethesda (Md.) Experiment Station of the Bureau of Animal Industry, all in healthy condition and apparently normal. The animals were kept under observation long enough before experimental use to exclude any but the most remote possibilities of accidental factors.

PREPARATIONS TESTED

The preparations of echinacea employed in the remedial work consisted of the following:

1. A sample of "Specific Medicine Echinacea," manufactured by and obtained from Lloyd Brothers, of Cincinnati, Ohio. This is a liquid preparation which is stated to contain 480 gr. of echinacea root per fluid ounce, or slightly more than a modern fluid extract. It contained 69 per cent of alcohol and conformed to the organoleptic tests for select echinacea. It was identified and preserved free from change during the whole course of the investigation. This remedy was diluted with distilled water for administration per os. The treatment caused the mixture to become cloudy because of the suspension of the resinous and oily constituents of the plant. These mixtures were never allowed to stand long enough for the insoluble matters to separate but were given to the animals while still in the stage of emulsion. In this way it is certain that the guinea pigs received all of the constituents of echinacea which are soluble in 69 per cent alcohol.

2. A fluid extract of echinacea purchased on the open market. This contained 70 per cent of alcohol and was identified, preserved, and administered exactly as was the specific medicine mentioned above.

3. "Subculoyd Inula and Echinacea," manufactured by and obtained from Lloyd Brothers. This liquid was used in the greater portion of the parenteral administrations. It is stated to contain, in 3 mils, 1.33 mils of *Inula helenium* and 1 mil of echinacea. It does not contain alcohol. This material was scrupulously preserved from contamination and change. In certain of the experiments it was administered intra muscularly; in other cases it was injected subcutaneously. Upon

autopsy of animals treated with this liquid there was noticed some necrosis of the tissues at the points of injection, but no other unfavorable results from its administration were observed.

Certain other preparations of echinacea which are sometimes used were not tested. A tincture of the green root is on the market, as is also a variety of powdered and solid extracts of echinacea. These preparations are all made with a menstruum of strong alcohol, and it is therefore not to be supposed that they contain any components not present in the fluid extracts which we used. The manufacturers of certain green-root tinctures assert that this product is superior to preparations of the dried root; there is, however, not the slightest published evidence to substantitate this assertion. The early settlers are said to have used the green root bruised and in the form of infusion. In the present work no such form of the remedy was used. It is quite possible that an infusion would contain some substances which are absent in the strongly alcoholic preparations and might, on this account, affect the organism differently. The claims of the therapeutic efficiency of echinacea have, however, been very largely made through the use of alcoholic preparations, and we therefore felt justified in employing these in determining its value as a remedy.

PATHOLOGICAL CONDITIONS TREATED

The acute experimental pathological conditions produced in the guinea pigs were tetanus, botulism (in both of which the diseases were produced by bacterial toxins), anthrax, septicemia (in both of which the bacteria were injected into the animals), and crotalus poisoning (in which the venin of rattlesnakes was injected). The chronic conditions were those of tuberculosis, which was produced by inoculation with the bacillus, and a trypanosomiasis (dourine), produced by inoculation with the trypanosomes. The sources of these materials and the methods of injection are described in the part of this paper which reports the experimental work.

METHODS

The methods employed for testing the remedial powers of echinacea against these several conditions were as follows:

1. Animals were injected with the pathogenic material and were immediately afterwards treated with echinacea, in suitable doses, one dose per diem, until the animal succumbed or became unable to swallow (if the administration was per os).

2. Animals were dosed with echinacea for several days before they were injected with pathogenic material, a protective treatment designed to favor the drug as much as possible, and were given remedial doses as long after the injection as possible. The treatment with the "Subculoyd" followed the same course. Treatment was necessarily suspended on Sundays and holidays, but in all except the chronic cases the time was so chosen as to minimize breaks due to such cause.

DOSAGE

The dose of fluid extract echinacea is variously given as from 10 minims to 0.5 fluid ounce for adult human beings, and for the "Subculoyd" preparation the parenteral dose recommended is 3 to 10 mils daily. It has also been stated that large doses of echinacea do not produce toxic effects upon healthy subjects, although this has been contradicted. The doses chosen for our experimental animals ranged from 0.25 to 1 mil daily of fluid extract and from 0.2 to 0.5 mil daily of subculoyd, which, calculated on a kilogram-of-body-weight basis, would correspond to from 40 to 160 mils daily of fluid extract and from 30 to 60 mils daily of the "Subculoyd" for man. It is well known, however, that to produce a given effect in guinea pigs requires very much larger doses per kilo than in larger animals. We decided upon a large dose of the remedy so as to favor the echinacea as much as possible and to remove any possibility of failure through administration of inadequate amounts.

GENERAL RESULTS AND CONCLUSIONS

In no one of the diseases treated with echinacea was any evidence obtained to show that the plant exerts any influence upon the course of infectious processes under laboratory conditions. Daily feeding of animals with echinacea preparations for several days before injection of microorganisms or their toxins did not increase the resistance of the animals to these agents. In the two chronic cases where the animals were given doses of echinacea preparations for extended periods of time nothing appeared in the autopsy pictures which could be attributed to the action of the echinacea per se, except that in two cases a gastric catarrh was present which may have been due to this plant. In all cases the course of the disease was the same in the control animals and in the animals which were given remedial treatment.

It does not appear, therefore, that echinacea or the preparation of inula and echinacea are of value in the treatment of diseases produced by microorganisms and their toxic products.

EXPERIMENTAL WORK

I.—TESTS OF ECHINACEA AS A REMEDY FOR TETANUS

In order to test the efficacy of echinacea as a remedy for tetanus a total of 29 guinea pigs was used. The animals were injected with a sample of standard tetanus toxin furnished by the Hygienic Laboratory of the United States Public Health Service. This material was kindly placed at our disposal by Dr. W. N. Berg, of our laboratory, who had used a part of it in his work on the destruction of tetanus antitoxin by chemical agents (*2*). It had been carefully standardized; the minimal

lethal dose was 0.0007 mgm. for a 350-gm. guinea pig. The material was preserved in vacuo in the dark and at low temperature. A fresh solution of the toxin was prepared for use by carefully weighing out a small quantity and dissolving this in just enough sterile normal salt solution to furnish a liquid which should contain 6 minimal lethal doses per mil. Each of the experimental animals received 0.5 mil of this solution, an equivalent of 3 minimal lethal doses.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Four guinea pigs were each given a 3-mil dose of a mixture of 1 mil of the "Specific Medicine Echinacea" and 2 mils of distilled water once a day for six days, a total of 6 mils of the remedy. The animals were rested one day and on the eighth day were given another dose of the remedial mixture, immediately followed by a subcutaneous injection of 0.5 mil of tetanus toxin solution (3 minimal lethal doses). On the following day all the animals received a dose of the remedy, so that each guinea pig had then received a total of 8 mils of specific echinacea, equivalent to somewhat more than 8 gm. of the root.

All of the animals exhibited the typical symptoms of tetanus and died on the ninth day. The autopsies were negative; no evidence of any ntercurrent disease was obtained. Three control guinea pigs which were injected at the same time as the experimental animals died on the same day with symptoms of tetanus and furnished the same post-mortem

EXPERIMENT 2.—ECHINACEA INJECTED INTRAMUSCULARLY

Echinacea injected intramuscularly was tested upon five guinea pigs. The undiluted "Specific Medicine Echinacea" was injected into the right and left thighs on alternate days. Each animal received four 0.5-mil doses, one per day, a total of 2 mils. The treatment caused considerable swelling at the points of injection. On the fourth day the animals were all given subcutaneous injections of 0.5 mil of the tetanus toxin solution. They all exhibited the characteristic symptoms of tetanus and died early in the morning of the third day after the injection. The autopsy showed considerable local reaction of the tissues to the injection of the echinacea. This consisted of a sero-sanguineous infiltration of the subcutaneous and muscular tissues with small areas of degeneration in the musculature at the point of injection. The internal organs showed no gross lesions.

EXPERIMENT 3.—ECHINACEA AND TOXIN ADMINISTERED SIMULTANEOUSLY

In order to determine whether echinacea possesses properties similar to the antitoxins, five guinea pigs were injected subcutaneously with 0.5 mil of the tetanus toxin solution and immediately received 0.5 mil of undiluted "Specific Medicine Echinacea" injected intramuscularly into

the right thighs. On the following day 0.5 mil of the remedy was injected into the left thighs of the animals. This treatment was wholly remedial, no protective doses having been given as in experiments 1 and 2 of this series. In three days after the injection of the toxin all the animals were dead after exhibiting typical tetanus. The autopsy picture was similar to that in experiment 2.

EXPERIMENT 4.—INULA AND ECHINACEA INJECTED INTRAMUSCULARLY

Protective doses of the "Subculoyd Inula and Echinacea" were injected intramuscularly into five guinea pigs. The dose administered was 0.5 mil per day for six days, a total of 3 mils, corresponding to 1 gm. of echinacea and 1.33 gm. of inula. On the eighth day after the treatment was begun the animals were injected with 0.5 mil of tetanus toxin solution, and a dose of 0.5 mil "Subculoyd" was given. The total dose of the remedy was 3.5 mils. On the following day all the guinea pigs showed typical symptoms of tetanus, and one died; the remaining four died the next day. On autopsy there was found a moderately severe local reaction of the tissues to the injection of the inula and echinacea. The subcutaneous and muscular tissues at the site of injection showed considerable hemorrhage and sero-sanguineous infiltration. No gross lesions were apparent in any of the internal organs.

EXPERIMENT 5.—INFLUENCE OF ALCOHOL ON TETANUS

Since the "Specific Medicine Echinacea" employed in the foregoing experiments contained 69 per cent of ethyl alcohol, it was considered desirable to study the influence of this factor upon tetanus under the conditions of the echinacea experiments. Accordingly, a mixture of alcohol and distilled water was made which contained exactly 69 per cent of alcohol, and this was injected intramuscularly into four guinea pigs in 0.5-mil doses. Each guinea pig received two doses, one into the right thigh and, on the next day, one into the left thigh. Two days afterwards all four received 0.5 mil of tetanus toxin solution subcutaneously. In three days two of these animals died, and the remaining two died during the following night. All showed typical symptoms of tetanus. The autopsy showed some congestion of the subcutaneous tissues at the points of injection of the alcohol, hemorrhage in the musculature, and evidence of local degeneration of the muscles. No gross lesions were apparent in any of the internal organs.

EXPERIMENT 6.—CONTROLS

The six control animals were kept under the same conditions as the experimental animals and received the same amounts of tetanus toxin. They all developed the typical symptoms of tetanus and died in less

than three days. The autopsies were negative. No evidence of an intercurrent disease was obtained.

The results of this series of experiments are given in Table I.

TABLE I.—*Results of experiments with echinacea in the treatment of tetanus*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Dose of toxin.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*	*M. l. d.* [a]			
1.....	18	395	7	3	Tetanus.....	Died.....	2.
	19	380	7	3	.....do.....	...do.....	2.
	21	335	7	3	.....do.....	...do.....	2.
	22	370	7	3	.....do.....	...do.....	2.
2.....	1	445	2	3	.....do.....	...do.....	Less than 3.
	2	455	2	3	.....do.....	...do.....	Do.
	3	490	2	3	.....do.....	...do.....	Do.
	4	440	2	3	.....do.....	...do.....	Do.
	5	445	2	3	.....do.....	...do.....	Do.
3.....	13	455	1	3	.....do.....	...do.....	3.
	14	470	1	3	.....do.....	...do.....	
	15	435	1	3	.....do.....	...do.....	
	16	450	1	3	.....do.....	...do.....	
	17	460	1	3	.....do.....	...do.....	
4.....	26	490	4	3	.....do.....	...do.....	3.
	27	470	4	3	.....do.....	...do.....	3.
	28	455	4	3	.....do.....	...do.....	3.
	29	400	4	3	.....do.....	...do.....	4.
	30	445	4	3	.....do.....	...do.....	2.
5.....	9	590	1	3	.....do.....	...do.....	4.
	10	480	1	3	.....do.....	...do.....	4.
	11	410	1	3	.....do.....	...do.....	3.
	12	390	1	3	.....do.....	...do.....	3.
6 (controls).	6	415	0	3	.....do.....	...do.....	Less than 3.
	7	480	0	3	.....do.....	...do.....	Do.
	8	440	0	3	.....do.....	...do.....	Do.
	23	475	0	3	.....do.....	...do.....	2.
	24	435	0	3	.....do.....	...do.....	2.
	25	395	0	3	.....do.....	...do.....	2.

[a] Minimal lethal dose.

SUMMARY OF EXPERIMENTS WITH TETANUS

"Specific Medicine Echinacea" was administered to guinea pigs both per os and intramuscularly, the "Subculoyd Inula and Echinacea" was administered to guinea pigs intramuscularly, and 69 per cent alcohol was injected intramuscularly into guinea pigs, as a means of treatment for tetanus. All of these animals were injected with 3 minimal lethal doses of standard tetanus toxin in solution, some animals being injected several days after they had been treated with echinacea, while others were injected first and then treated with echinacea. Neither the protective treatment nor the remedial treatment nor a combination of the two appeared to influence the course of the disease, as all the experimental animals acted in the same way and died in the same time as the controls. From these results it does not appear that echinacea possesses remedial value against experimental tetanus in laboratory animals.

II.—TESTS OF ECHINACEA AS A REMEDY FOR BOTULISM

Since echinacea did not appear to influence the action of tetanus toxin, it was thought desirable to compare its action against another bacterial toxin. For this purpose botulinus toxin was chosen. The material used to produce botulism in the experimental animals consisted of a germ-free filtrate of a glucose beef infusion culture of *Bacillus botulinus* (Boise strain) (*17*) incubated for one month at 37° C. The filtrate was diluted with sterile normal salt solution in such amount that 1 mil was equivalent to very nearly 10 minimal lethal doses for a 400-gm. guinea pig. This toxin was not injected into the animals but was fed through the mouth in order to duplicate the conditions under which this type of poisoning usually occurs.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Three guinea pigs only were used, because the results of the experiment were so free from uncertainty that it was not considered necessary to sacrifice more animals in order to determine the facts. The animals were all given 2-mil doses daily of a mixture of 0.5 mil fluid extract echinacea and 1.5 mils distilled water for 6 days. The total protective dosage was 3 mils of the fluid extract, equivalent to 3 gm of echinacea. The animals were rested one day, and on the eighth day after the beginning of the experiment all received 1 mil (10 minimal lethal doses) of botulinus toxin immediately after receiving a 2-mil dose of the remedial mixture. On the following day all the animals were sick. No. 78 received a remedial dose of 2 mils of the echinacea mixture. No. 79 received 1 mil of the same mixture, which was all that it could swallow. With No. 80 the symptoms of pharyngeal paralysis were so marked that it was considered inadvisable to drench the animal on account of the danger of strangulation. This animal died during the afternoon. The remaining two were found dead in the morning of the second day after. The treated pigs and the controls showed no differences. The autopsy showed general hyperemia of the internal organs; there was no evidence of any intercurrent disease.

EXPERIMENT 2.—CONTROLS

Two guinea pigs were used as controls. These animals were fed a 1-mil dose of botulinus toxin (10 minimal lethal doses) on the same date as the experimental pigs. In about 18 hours both animals showed symptoms of botulism; one died in 23 hours after the dose; the other was found dead in the morning of the third day after the dose. The post-mortem findings were similar to those for the experimental animals. The results are summarized in Table II.

TABLE II.—*Results of experiments with echinacea in the treatment of botulism*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Dose of toxin.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*	*M. l. d.*			
1.....	78	345	4	10	Sick.....	Died.....	3
	79	370	3.75	10	.....do.....	.....do.....	3
	80	405	3.5	10	.....do.....	.....do.....	1
2 (controls).	81	390	0	10	.....do.....	.....do.....	3
	82	365	0	10	.....do.....	.....do.....	1

SUMMARY OF EXPERIMENTS WITH BOTULISM

Fluid extract echinacea was administered per os to guinea pigs for a total of six protective doses. The animals were then fed botulinus toxin. The treatment with echinacea was continued as long as the animals were able to swallow. All the experimental animals developed positive symptoms of botulism and died within three days after ingesting the toxin. From this it does not appear that echinacea possesses remedial value against botulism.

III.—TESTS OF ECHINACEA AS A REMEDY FOR SEPTICEMIA

Twelve guinea pigs were used in testing the remedial value of echinacea in septicemia. The pathogenic material was a 48-hour-old glycerin-agar culture of *Bacillus bovisepticus* of only moderate virulence for laboratory animals. A faintly cloudy suspension of the organisms in sterile normal salt solution was prepared and used for inoculation. While no attempt was made to determine the minimal lethal dose of this organism for guinea pigs, a few preliminary tests undertaken indicated that the dose employed in the following experiments was not excessive.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS (PROTECTIVE)

In order to determine whether the administration of echinacea would increase the resistance of the organism to septicemia if given sufficient time to develop immunity, two guinea pigs were given four daily doses of 3 mils of a mixture of 1 mil of "Specific Medicine Echinacea" and 2 mils of distilled water. The total protective dose was 4 mils, all administered per os. The animals were then allowed to rest for 11 days, when they were injected subcutaneously with 0.5 mil *Bacillus bovisepticus* suspension. Both animals became sick; one died in three days and the other in five days. Two of the controls died in three days and the third control survived. The autopsy showed septicemia.

EXPERIMENT 2.—ECHINACEA ADMINISTERED PER OS (REMEDIAL)

Two guinea pigs were given two daily doses per os of 3 mils of the diluted echinacea mixture used in experiment 1. On the third day they were injected with 0.5 mil of *Bacillus bovisepticus* culture, and immediately afterwards were given a 3-mil dose of the echinacea mixture per os. On the following day both animals were very sick. They were given a fourth dose of 3 mils of the echinacea mixture per os. The total dose was 4 mils of specific echinacea, equal to 4 gm. of the root. Case 65 died in 24 hours and case 66 in 48 hours. The autopsy showed septicemia; typical organisms were demonstrated in blood and organs.

EXPERIMENT 3.—INULA AND ECHINACEA INJECTED INTRAMUSCULARLY (PROTECTIVE)

This experiment was conducted exactly like experiment 1 of this series except that the "Subculoyd" preparation was used instead of the "Specific Medicine Echinacea." Three guinea pigs were given four daily doses of the "Subculoyd" preparation, 0.5 mil being injected intramuscularly, first into the right and then into the left thigh. The total dose was 2 mils. The animals were allowed to rest 11 days and then were injected subcutaneously with 0.5 mil of *Bacillus bovisepticus* culture. All became sick. Case 62 died in 6 days after the inoculation, case 64 died in 12 days, and case 63 survived, being discharged as recovered 10 weeks after the injection. The autopsies on the fatal cases revealed typical pictures of septicemia, and the organisms were demonstrated in the blood and organs.

EXPERIMENT 4.—INULA AND ECHINACEA INJECTED INTRAMUSCULARLY (REMEDIAL)

Three guinea pigs were given daily injections of the "Subculoyd" preparation, the injections being made alternately into the right and left thighs. The dose used was 0.5 mil. After the third injection the animals were all inoculated subcutaneously with 0.5 mil of *Bacillus bovisepticus* culture. On the following day the animals were given a fourth dose of the "Subculoyd." The total dose of remedy was 2 mils. All these cases succumbed to the infection, the first in one day, the second in two days, and the third in three days after the inoculation. The autopsies showed the typical septicemia pictures, and the organisms were demonstrated in the blood.

EXPERIMENT 5.—CONTROLS

Three control animals, each inoculated subcutaneously with 0.5 mil of *Bacillus bovisepticus* culture, all became sick, and two succumbed to the infection. The third survived and after 10 weeks' observation was discharged as recovered. The autopsies on the fatal cases showed septicemia, and the organisms were demonstrated in the blood and organs.

The experiments for septicemia are summarized in Table III.

TABLE III.—*Results of experiments with echinacea in the treatment of septicemia*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Dose of culture.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*	*Mil.*			
1	60	540	4	0.5	Sick	Died	5
	61	500	4	.5	do	do	3
2	65	385	4	.5	do	do	1
	66	335	4	.5	do	do	2
3	62	485	2	.5	do	do	6
	63	505	2	.5	do	Recovered	
	64	385	2	.5	do	Died	12
4	67	355	2	.5	do	do	3
	68	425	2	.5	do	do	1
	69	300	2	.5	do	do	2
5 (controls)	70	380	0	.5	do	Recovered	
	71	365	0	.5	do	Died	3
	72	355	0	.5	do	do	3

SUMMARY OF EXPERIMENTS WITH SEPTICEMIA

"Specific Medicine Echinacea" and "Subculoyd Inula and Echinacea" were used as protective and as remedial measures against septicemia induced by *Bacillus bovisepticus*. The attempt was made to immunize animals against septicemia by administration of the echinacea preparations several days before inoculation. In no case did it appear that echinacea either increased the resistance of the organism to the infection or served to modify it when given as a remedy.

IV.—TESTS OF ECHINACEA AS A REMEDY FOR ANTHRAX

The pathogenic material used to produce anthrax in the experimental animals was a faintly cloudy suspension of *Bacillus anthracis* (48-hour-old agar culture) in sterile normal salt solution. The remedial action of the fluid extract only was investigated, and only five experimental animals were used, the results of the experiment being so definite as not to necessitate the sacrifice of any more animals.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Three pigs were given daily doses per os of 2 mils of fluid extract echinacea diluted with 1.5 mils distilled water for 6 days. The total protective dose was 3 mils, equal to 3 gm. of echinacea root. On the eighth day the animals were given per os the same dose of echinacea and immediately afterwards were inoculated with 0.4 mil of anthrax material subcutaneously. On the following day they were given a second remedial dose of echinacea. The total echinacea given was 4 mils of fluid extract. All the animals became sick and all succumbed. No evidence was obtained that echinacea has any influence upon the course of anthrax in experimental animals. The autopsy was typical for anthrax; organisms were demonstrated microscopically in the blood.

EXPERIMENT 2.—CONTROLS

Two controls were chosen at the beginning of experiment 1 of this series and were kept under observation for 8 days, when they were injected subcutaneously with 0.4 mil of the anthrax material at the same time as the experimental animals. Both controls became sick, and one died in 4 and the other in 8 days, having survived the experimental guinea pigs by 1 and 5 days, respectively. The autopsy showed typical anthrax.

Table IV summarizes the results of the experiments for anthrax.

TABLE IV.—*Results of experiments with echinacea in the treatment of anthrax*

Experiment No.	Guinea pig. No.	Weight of guinea pig.	Total dose of remedy.	Dose of culture.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*	*Mil.*			
1	73	440	4	0.4	Sick.....	Died.....	3
	74	285	4	.4	.....do.....	.....do.....	3
	75	450	4	.4	.....do.....	.....do.....	3
2 (controls)	76	355	0	.4	.....do.....	.....do.....	8
	77	350	0	.4	.....do.....	.....do.....	4

SUMMARY OF EXPERIMENTS WITH ANTHRAX

Experimental animals were given protective and remedial doses of fluid extract echinacea and were inoculated with *Bacillus anthracis*. All the animals died, those which were treated dying before the control animals. Echinacea does not appear to be of value as a remedy for anthrax.

V.—TESTS OF ECHINACEA AS A REMEDY AGAINST RATTLESNAKE VENIN

Twenty-five guinea pigs were used in the experiments with rattlesnake venin. The venin was furnished by Dr. Park Findley, of Des Moines, Iowa, who had obtained it while with the United States Army on the Mexican border. The venomous secretion of the rattlesnake was collected and dried by inspissation in the sun. This treatment, of course, somewhat attenuated the venin. The product occurred in brittle, clear, yellowish granules, much resembling dried egg albumen. The minimal lethal dose was determined as 2 mgm. for a 400- to 450-gm. guinea pig. The venin was hemolytic in a dilution of 1 to 1,000 against washed sheep corpuscles. For injection, a quantity of the venin was carefully weighed out and dissolved in sufficient sterile normal salt solution to furnish a liquid which would contain 2 mgm. per mil.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Each of three guinea pigs received daily 3 mils of a mixture of 1 mil "Specific Medicine Echinacea" and 2 mils water for two doses, a total of 2 mils echinacea, as protective treatment. On the third day the animals were given 2 mgm. of rattlesnake venin in 1 mil of salt solution injected

subcutaneously into the ventral abdominal wall, and immediately afterwards a dose of the echinacea was given per os. No. 50 was found dead on the following morning. The surviving pigs were given a dose of the echinacea mixture. The total amount of echinacea given in the first case was 3 mils; in the second and third cases it was 4 mils. These latter guinea pigs died on the third day after the injection of the venin. All the animals showed the characteristic symptoms and local lesions of this type of poisoning. On autopsy, the characteristic local lesions were found, consisting of a marked inflammatory swelling with necrosis and sloughing of the skin over a considerable area surrounding the point of injection. In cases of early death from rattlesnake poisoning there is usually some oozing of dark, incoagulable blood from the wound at the seat of injection and extensive extravasation of blood into the subcutaneous and muscular tissues. The inflammatory process in most cases extends through the abdominal wall and involves the peritoneum. If the animal survives for several days there may be complete sloughing of the abdominal wall, allowing the viscera to protrude. The internal organs are usually grossly normal in appearance, except in the case of the kidneys, which may be somewhat enlarged and congested with evidence of parenchymatous degeneration.

EXPERIMENT 2.—INULA AND ECHINACEA INJECTED INTRAMUSCULARLY

Each of three guinea pigs received 0.5 mil of the "Subcoloyd Inula and Echinacea" in the right thigh on the first day; on the second day the same dose was injected into the left thigh, both injections being made deeply into the gluteal muscles. The total protective dose was 1 mil. On the third day 1 mil of the venin solution, equal to 2 mgm. of dry venin, was injected subcutaneously into the belly, and immediately afterwards 0.5 mil of "Subculoyd" was injected into the right thigh. On the following day all the animals showed the characteristic symptoms and 0.5 mil of the "Subculoyd" was injected into the left thigh of each animal. The total dose was 2 mils. On the third day No. 51 died; on the fifth day No. 53 died; and six weeks later No. 52 was discharged as recovered. The autopsy was the same as in experiment 1 of this series. The guinea pigs showed the usual local lesions produced by the injection of the inula and echinacea.

EXPERIMENT 3.—CONTROLS

Three guinea pigs were used as controls and were injected subcutaneously into the belly with 1 mil of venin solution, corresponding to 2 mgm. of dry venin. All the controls were sick on the following day. No. 57 and 58 died on the second day and No. 59 on the third day after the injection of the venin. The autopsy showed the same conditions as in experiment 1 of this series. There was no apparent difference between the controls and the treated animals in experiments 1 and 2.

The results are given in Table V.

TABLE V.—*Results of experiments with echinacea as a remedy against rattlesnake venin*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Dose of venin.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*	*Mgm.*			
A[a]	31	370	0	0.1	Sick	Recovered	
	32	390	0	.2	do	do	
	33	290	0	.3	do	do	
	34	297	0	.4	do	do	
	35	315	0	.5	do	Died	28
	36	260	0	.6	do	Recovered	
	37	350	0	.6	do	Died	2
	38	290	0	.7	do	Recovered	
	39	265	0	.7	do	Died	33
	40	440	0	.5	do	do	24
	41	340	0	.5	do	do	28
	42	.....	0	1.0	do	Recovered	
	43	.....	0	1.0	do	do	
	44	.....	0	2.0	do	Died	3
	45	.....	0	2.0	do	do	3
1	48	395	4	2.0	do	do	3
	49	400	4	2.0	do	do	3
	50	370	3	2.0	do	do	1
2	51	440	2	2.0	do	do	3
	52	425	2	2.0	do	Recovered	
	53	360	2	2.0	do	Died	5
3 (controls).	57	385	0	2.0	do	do	2
	58	405	0	2.0	do	do	2
	59	405	0	2.0	do	do	3

[a] To test toxicity of venin.

SUMMARY OF EXPERIMENTS WITH RATTLESNAKE VENIN

"Specific Medicine Echinacea" was administered to guinea pigs per os, and "Subculoyd Inula and Echinacea" was injected as a means of treatment against the venin of the rattlesnake. The venin had been standardized and the minimal lethal dose determined. Neither of the echinacea preparations appeared to influence the course of the poisoning. From these results it does not appear that echinacea is of value in the treatment of rattlesnake poisoning in experimental animals under laboratory conditions.

VI.—TESTS OF ECHINACEA AS A REMEDY FOR TUBERCULOSIS

It has often been asserted that echinacea is a cure for tuberculosis, and for this reason tuberculosis was chosen as one of the chronic diseases upon which to test the remedial value of the plant. The type of organism used to inoculate the experimental animals was strictly human (Igoe strain). The immediate material used for our purpose was one-third of a tuberculous spleen from a guinea pig, third passage of the original material, finely triturated in mortar and suspended in 10 mils of normal salt solution. The dose was 1 mil per guinea pig, injected intraperitoneally.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Three guinea pigs were inoculated with tubercle bacilli November 20, 1919, and on the following day treatment was begun. Each animal received a dose of a mixture of 0.25 mil of fluid extract echinacea and 0.75 mil distilled water per os each week day. The animals were weighed three times a week. All the animals showed a progressive loss in weight (see Table VII) and eventually succumbed. Case 85 died December 10, (20 days after inoculation), after having received a total of 3.5 mils of fluid extract echinacea as a remedy. The autopsy in this case was negative. Case 84 died December 22 (32 days after inoculation), having received 6 mils of the echinacea. Case 86 was found dead in the morning of December 26 (36 days after inoculation), having received 6.75 mils of the echinacea. In the last two cases the autopsies revealed the typical picture of generalized tuberculosis.

As these were probably the first experimental animals which had ever received echinacea over an extended period of time, it was interesting to observe the effects of the plant on the animals themselves, and especially upon the gastrointestinal tract. In case 85 there was found a chronic catarrhal gastritis which was absent in cases 83 and 84. Apart from the tubercular lesions there was no abnormality found in the other organs upon macroscopic examination.

EXPERIMENT 2.—INULA AND ECHINACEA INJECTED SUBCUTANEOUSLY

Three guinea pigs were inoculated with the tuberculous material as in experiment 1 on November 20, 1919, and the treatment was begun on the following day. Each animal received subcutaneously 0.2 mil of the "Subculoyd Inula and Echinacea" each week day and was weighed three times a week. All showed progressive loss of weight, as shown in Table VII. Case 87 died December 19, 29 days after inoculation, after having received 4.2 mils of the remedy. Case 88 died December 23, 33 days after inoculation, having received 5 mils of the remedy, and case 86 died on December 28, 38 days after inoculation, having received 5.8 mils of the remedy. The autopsies in these cases showed great emaciation, some necrosis at the points of injection, and generalized tuberculosis. There was no evidence of systemic effects from the remedy.

EXPERIMENT 3.—CONTROLS

Two control guinea pigs were inoculated with the same tuberculous material as the animals in experiments 1 and 2, on November 20, 1919, and were weighed three times a week. They lost weight (see Table VII). Case 89 died December 3, 13 days after inoculation, and case 90 died December 23, 33 days after inoculation. The autopsy showed generalized tuberculosis.

The experiments are summarized in Table VI.

TABLE VI.—*Results of experiments with echinacea in the treatment of tuberculosis*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*			
1	83	430	6. 75	Sick	Died	36
	84	425	6. 00	do	do	32
	85	495	3. 50	do	do	20
2	86	470	5. 80	do	do	38
	87	450	4. 20	do	do	29
	88	405	5. 00	do	do	33
3 (controls)	89	425	0	do	do	13
	90	550	0	do	do	33

TABLE VII.—*Progressive loss of weight of guinea pigs in experiments with echinacea in the treatment of tuberculosis*

Date.	Weight of guinea pigs treated with fluid extract echinacea.			Weight of guinea pigs treated with "Subculoyd Inula and Echinacea."			Weight of control guinea pigs.	
	No. 83.	No. 84.	No. 85.	No. 86.	No. 87.	No. 88.	No. 89.	No. 90.
1919.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Nov. 18	430	425	495	475	450	405	425	550
20	410	430	480	455	450	405	415	535
22	395	410	470	440	420	380	405	520
24	405	410	490	460	425	360	395	530
26	400	415	475	460	415	370	370	525
28	390	415	480	470	415	370	325	525
Dec. 1	385	390	475	470	395	355	285	510
3	380	395	460	470	405	360	265	510
5	365	385	440	460	375	340	.....	510
8	355	365	405	465	360	335	.....	485
10	350	355	.....	460	355	325	.....	485
12	345	345	.....	445	325	330	.....	480
15	310	325	.....	405	285	300	.....	400
17	295	290	.....	405	260	280	.....	380
19	290	270	.....	400	.....	280	.....	355
22	270	.....	.....	355	.....	245	.....	305
24	240	.....	.....	340	.....	.....	.....	.....
26	.....	.....	.....	320	.....	.....	.....	.....

SUMMARY OF EXPERIMENTS WITH TUBERCULOSIS

Fluid extract echinacea was administered per os, and "Subculoyd Inula and Echinacea" was injected subcutaneously into experimental guinea pigs daily for extended periods as remedies for tuberculosis produced by a human type organism. Neither of the preparations appeared to influence the course of the disease. From these results it does not seem probable that either fluid extract echinacea or the "Subculoyd Inula and Echinacea" is of value in the treatment of tuberculosis.

The experimental animals did not show organic effects from echinacea ingested in large doses for a long time.

VII.—TESTS OF ECHINACEA AS A REMEDY FOR TRYPANOSOMIASIS (DOURINE)

In connection with the experimentation with tuberculosis it was considered of interest to study the remedial action of the echinacea preparations upon another chronic condition. Trypanosomiasis induced by *Trypanosoma equiperdum* and commonly called dourine was chosen. This disease as produced under laboratory conditions in guinea pigs runs a course of from 7 to 11 weeks, allowing ample time for the exhibition of quantities of remedial agents and consequently favoring the remedy more than a speedy, acute infection would.

The material used to produce the disease was kindly furnished by Dr. H. W. Schoening, of this laboratory. It consisted of a normal salt suspension of a sample of blood freshly drawn from rats which had been inoculated three days previously with *Trypanosoma equiperdum*. Upon microscopic examination this showed numbers of trypanosomes. The dose given was 0.5 mil, injected subcutaneously.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Three guinea pigs were inoculated with the dourine material on December 1, 1919. On the following day treatment was begun, each animal receiving 1 mil of a mixture of 0.25 mil fluid extract echinacea and 0.75 mil of distilled water. This dose was given each week day thereafter as long as the animal survived. All the animals were weighed three times a week. The weights are reported in Table IX. At intervals the blood of some of the animals was examined microscopically for the presence of trypanosomes; on December 17 these were demonstrated in the peripheral circulation of case 93, on January 6 in that of case 92, on January 16 in cases 91 and 92, and on March 3 in case 93. All the animals showed the typical symptoms of trypanosomiasis. Case 91 died on the sixty-first day, after having received 12.5 mils of the fluid extract; case 92 died on the sixty-fourth day after having receiving 13 mils of fluid extract; case 93 died on the ninety-third day, after having received 15.75 mils of fluid extract. Treatment of case 93 was suspended February 14. The autopsies showed the usual picture of this type of infection. In case 91 there was a chronic catarrhal gastritis; otherwise no organic effects from the extended ingestion of the echinacea were discovered.

EXPERIMENT 2.—INULA AND ECHINACEA INJECTED SUBCUTANEOUSLY

Three guinea pigs were inoculated and treated exactly as in experiment 1, except that the remedy given was 0.2 mil of the "Subculoyd Inula and Echinacea" each week day. The weights of the animals are given in Table IX. On December 17 trypanosomes were demonstrated in the peripheral circulation of case 94, on January 6 in that of case 96, and on

January 16 case 96 was positive, while cases 94 and 95 did not show trypanosomes. Case 96 died on the forty-eighth day after inoculation, having received 7.8 mils of the remedy; case 95 succumbed on the sixty-sixth day, after receiving a total of 10.8 mils of the remedy, and case 94 died on the seventy-first day, having received 11.4 mils of the remedy. The autopsies in these cases showed a dirty, dark discoloration of the subcutaneous and superficial abdominal muscular tissues over the area where injections were made. Extreme emaciation was evident, the spleen was greatly enlarged, and in general the typical dourine picture was present.

EXPERIMENT 3.—CONTROLS

Four guinea pigs were used as controls. These were inoculated on the same date as those in experiments 1 and 2 and were kept in separate cages. One animal died in 17 days, another died in 30 days, and the remaining 2 died in 78 and 79 days, respectively, all with typical dourine symptoms.

These experiments are reported in Table VIII.

TABLE VIII.—*Results of experiments with echinacea in the treatment of dourine*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*			
1	91	475	12.5	Sick	Died	61
	92	505	13.0	do	do	64
	93	445	15.75	do	do	93
2	94	435	11.4	do	do	71
	95	400	10.8	do	do	66
	96	560	7.8	do	do	48
3 (controls).	97	535	0	do	do	17
	98	460	0	do	do	30
	99	535	0	do	do	78
	100	500	0	do	do	79

TABLE IX.—*Progressive loss of weight of guinea pigs in experiments with echinacea in the treatment of dourine*

Date.	Weights of guinea pigs treated with fluid extract echinacea.			Weights of guinea pigs treated with "Subculoyd Inula and Echinacea."		
	No. 91.	No. 92.	No. 93.	No. 94.	No. 95.	No. 96.
1919.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Nov. 29	475	505	445	435	400	560
Dec. 1	475	505	450	445	410	570
3	485	510	465	455	415	585
5	475	500	465	440	425	560
8	480	505	470	445	420	555
10	480	510	475	450	415	570
12	500	520	505	445	440	565
15	495	515	500	450	425	565
17	485	510	500	445	430	565
19	490	520	525	455	445	550
22	485	510	490	410	460	560
24	465	505	475	410	435	535
26	450	495	470	430	440	495
29	460	480	465	420	435	500
31	460	485	465	405	430	485
1920.						
Jan. 2	450	470	470	410	445	480
3	455	475	470	420	455	480
5	465	485	500	435	465	480
7	450	470	485	415	450	470
9	455	465	500	430	460	460
12	435	455	510	445	465	435
14	415	435	495	420	440	395
16	410	430	500	410	450	365
19	395	435	520	365	465	.....
21	385	415	505	340	445	.....
23	400	415	515	325	440	.....
26	390	385	510	305	440	.....
28	385	355	515	315	440	.....
30	.....	320	500	315	450	.....
Feb. 2	.....	.....	495	295	405	.....
4	.....	.....	480	295	.....	.....
6	.....	.....	490	305	.....	.....
9	.....	.....	455	.....	.....	.....
11	.....	.....	440	.....	.....	.....
13	.....	.....	450	.....	.....	.....
16	.....	.....	455	.....	.....	.....
19	.....	.....	455	.....	.....	.....
21	.....	.....	415	.....	.....	.....
24	.....	.....	390	.....	.....	.....
27	.....	.....	370	.....	.....	.....
Mar. 1	.....	.....	345	.....	.....	.....

SUMMARY OF EXPERIMENTS WITH DOURINE

Fluid extract echinacea and "Subculoyd Inula and Echinacea" were tested as remedies in trypanosomiasis (dourine). Neither of these preparations appeared to influence the course of the disease. They certainly have no curative value.

GENERAL SUMMARY

Various preparations of echinacea—namely, the "Specific Medicine Echinacea," the fluid extract echinacea, and the "Subculoyd Inula and Echinacea"—were studied as remedies in several types of infectious and allied diseases, both acute and chronic, in guinea pigs.

In both tetanus and botulism produced by the administration of bacterial toxin the course of the disease was not modified by the echinacea.

In septicemia produced by injection of a culture of *Bacillus bovisepticus*, and in anthrax produced by injection of *B. anthracis* the results indicated that echinacea had no influence.

In poisoning by the venin of the rattlesnake produced by injection of a solution of the dry venom the echinacea preparations were without curative effect.

In the chronic diseases, tuberculosis produced by injection of a human strain of the bacillus and trypanosomiasis produced by injection of *Trypanosoma equiperdum* the remedy was exhibited over an extended period of time without apparently influencing the course of these diseases.

Definite evidence of organic effects from the echinacea itself was not obtained.

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INVESTIGATIONS OF THE GERMICIDAL VALUE OF SOME OF THE CHLORIN DISINFECTANTS

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SCOPE OF THE INVESTIGATION

During the great World War, which from the surgical standpoint was distinguished by the frequency and intensity of wound infections, no class of antiseptics was more extensively employed than the so-called "chlorin antiseptics." When properly used they proved to be of very great value, as may be seen by a perusal of the various publications of Carrel and his colleagues and especially the book by Carrel and Dehelly (*2*).[1]

In view of the great amount of work already done on the value of these antiseptics in surgery no attempt has been made by the writer to cover that field of work. The experiments herein described were intended to furnish information regarding the value of the chlorin antiseptics for general disinfection. The members of this group actually tested were: (1) chloramin T, (2) Dakin's solution (NaOCl), (3) eusol (HOCl), and (4) chlorin.

"Chloramin T" is the abbreviated name given by Dakin to sodium-toluene-sulphon-chloramid (*4*). It is described as a "white crystalline solid with a faint chlorous odor" containing 12.6 per cent of chlorin and readily soluble in water. The material used in the present work was obtained under the trade name "Chlorazene." Its appearance corresponds to the foregoing description, and titration of an aqueous solution with potassium iodid and sodium thiosulphate showed it to contain 25 per cent of "available chlorin," which corresponds to 12.5 per cent of actual chlorin, since according to Dakin and Dunham (*5*) one molecule of chloramin T liberates two atoms of iodin. The explanation they give is that each atom of chlorin in chloramin T is equivalent to a molecule of hypochlorous acid, which liberates two atoms of iodin from an acidified iodid solution.

[1] Reference is made by number (italic) to "Literature cited," p. 110.

The term "Dakin's solution" as used in this paper signifies a neutral solution of sodium hypochlorite. The methods of preparation were essentially those given by Dakin and Dunham (*5*).

The details of the method with sodium carbonate are, according to Dakin and Dunham, as follows: One hundred and forty gm. of dry sodium carbonate (Na_2CO_3), or 400 gm. of the crystallized salt, are dissolved in 10 liters of water, and 200 gm. of bleaching powder containing 24 to 28 per cent of "available chlorin" are added. The mixture is very thoroughly shaken, and after it has stood half an hour the supernatant fluid is siphoned off from the precipitate and filtered through a cotton plug or through paper. Forty gm. of boric acid are added to the filtrate, and it is then ready for use.

The details of the method with sodium carbonate and sodium bicarbonate are, according to Dakin and Dunham, as follows: Two hundred gm. of bleaching powder (containing 24 to 28 per cent of "available chlorin") are shaken well with 5 liters of water and allowed to stand for an hour or two. In a separate vessel 94 gm. of sodium carbonate and 86 gm. of sodium bicarbonate ($NaHCO_3$) are mixed with 5 liters of water, and this solution is added to the bleaching powder suspension. The mixture is well shaken and allowed to stand until the precipitate settles. The clear supernatant fluid is then siphoned off and filtered.

In actual practice the writer made the following modifications. The amount of Dakin's solution made up at any one time was always smaller than the amount indicated above, but the relative proportions of ingredients were the same. The bleaching powder was rubbed up in a mortar with a little water until it was of a creamy consistency. It was then transferred to a graduated flask or cylinder and made up to volume with more water. Dry sodium carbonate, or the solution of sodium carbonate and sodium bicarbonate, was added in accordance with the directions of Dakin and Dunham, and their further directions were followed except that instead of the clear supernatant fluid being drawn off the entire mixture was shaken up and filtered through paper. The bleaching powder used contained approximately 28 per cent of "available chlorin."

In certain experiments Dakin's solution was also prepared by the direct action of chlorin upon a solution of sodium carbonate, with the use of the apparatus devised for the purpose by the Wallace & Tiernan Co., of New York City.

The term "eusol," as employed in this paper, signifies a solution prepared from bleaching powder in aqueous solution by the addition of an equal amount of boric acid. The originators of this solution (*12*) describe it as a solution of hypochlorous acid, but according to Dakin and Dunham (*5*) the solution is alkaline to litmus and contains a balanced mixture of calcium hypochlorite and calcium borate with an undetermined amount of free hypochlorous acid.

The method of preparation as given by Dakin and Dunham is as follows: To 1 liter of water add 12.5 gm. of bleaching powder and shake vigorously. Add 12.5 gm. of powdered boric acid and shake again. Allow the mixture to stand for some hours, preferably overnight, and then filter. In actual practice the writer made the following modifications: The bleaching powder was rubbed up in a mortar with a little water until the mixture had a creamy consistency. It was then transferred to a graduated flask or cylinder, the boric acid was added, and then the amount of water necessary to make up the volume. The mixture was shaken and then usually allowed to stand about two hours before it was filtered through paper.

Chlorin was used in these experiments in the form of an aqueous solution, standardized by titration with potassium iodid and sodium thiosulphate.

Dilutions of these various disinfectants were made up for test as follows: Chloramin T dilutions were made by weighing the solid and dissolving it in the required amount of water. In certain experiments a stock solution was made and titrated with potassium iodid and sodium thiosulphate, and dilutions of the stock solution were then made so as to contain specified amounts of "available chlorin."[1] But for the most part dilutions were made up to contain a given weight of the solid chloramin T.

Dakin's solution and eusol were prepared according to the directions previously given and were then titrated with potassium iodid and sodium thiosulphate. Dilutions were then made from these original solutions so as to contain a given amount of "sodium hypochlorite" or "hypochlorous acid" for Dakin's solution and eusol, respectively. In certain experiments the dilutions were made in both instances so as to contain given amounts of "available chlorin."

It has already been noted that according to Dakin and Dunham (*5*) eusol contains calcium hypochlorite with an indefinite amount of free hypochlorous acid. In a similar way Dakin's solution may contain not only sodium hypochlorite but also more or less hypochlorous acid, as stated by Cullen and Austin (*3*). As regards "available chlorin" Rosenau (*11*) states that this really represents available oxygen rather than available chlorin. All three terms, however, are convenient as conventional symbols and will be so used in this paper.

It should be stated further that for the purposes of certain experiments it was necessary to modify the methods of preparing Dakin's solution and eusol materially so as to secure more concentrated solutions. In all such instances the changes made are indicated in connection with the experiments.

[1] The quotation marks used in this and the following paragraph are intended to indicate that the terms are used in a conventional way for purposes of comparison, and not in their literal sense.

EXPERIMENTS WITH STAPHYLOCOCCUS AUREUS, BACILLUS PYOCYANEUS, AND B. TYPHOSUS AS TEST ORGANISMS

The cultures used in this series of experiments were stock cultures which had been carried along in this laboratory for some time. They had been previously examined in connection with other work and had been found true to type. The cultures of *Staphylococcus aureus* and *Bacillus pyocyaneus* were originally isolated from wounds and when received in this laboratory were virulent for guinea pigs and rabbits.

The first three experiments of this series were made with chloramin T only. The results are shown in Tables I and II.

EXPERIMENT 1.—This was a preliminary experiment to test the value of chloramin T against *Staphylococcus aureus* and *Bacillus typhosus*. The technic was as follows: One-tenth cc. of 24-hour bouillon culture was mixed with 25 cc. of blood serum.[1] Then 2.5 cc. of this mixture were mixed with 2.5 cc. of a dilution of chloramin T. After exposures of one hour and two hours, respectively, subcultures were made with a 3-mm. platinum loop into tubes of standard bouillon containing enough sodium thiosulphate to neutralize the disinfectant carried over.

The number of organisms present in the test mixtures were calculated to be 1,340,000 per centimeter for *Staphylococcus aureus* and 800,000 per centimeter for *Bacillus typhosus*. Dilutions given in the table are, of course, final dilutions, and the dilutions actually made up to begin with were naturally just twice as concentrated. The results are given in Table I.

TABLE I.—*Germicidal efficiency of chloramin T against Staphylococcus aureus and Bacillus typhosus, mixed with blood serum, using equal amounts of disinfectant and of serum plus culture* [a]

EXPERIMENT I

Concentration of chloramin T.	*Staph. aureus.*		*B. typhosus.*	
	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.
1 to 200	−	−	−	−
1 to 400	−	−	+	−
1 to 600	+	+	+	+
1 to 800	+	+	+	+

[a] + Signifies growth; −, no growth.

EXPERIMENTS 2 AND 3.—The technic used in these experiments was as follows: Each disinfectant dilution was mixed with an equal quantity of blood serum (2.5 cc. each of disinfectant dilution and serum), and to this mixture 2 drops of a 24-hour bouillon culture were added. The mixture was then vigorously shaken, and at intervals of 15 minutes, 1 hour, and 2 hours subcultures were made with a 3-mm. platinum loop

[1] Horse-blood serum was used in all experiments where blood serum is mentioned.

into tubes of standard broth. The mixtures were thoroughly shaken just before subcultures were made.

In these experiments and in all others described in this paper, the test mixtures were used at ordinary room temperatures.

The results of experiments 2 and 3 are given in Table II.

TABLE II.—*Germicidal efficiency of chloramin T against Staphylococcus aureus, Bacillus typhosus, and B. pyocyaneus when mixed with an equal quantity of blood serum before culture is added* [a]

EXPERIMENT 2

Concentration of chloramin T.	*Staph. aureus.*			*B. typhosus.*		
	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.
1 to 200	−	−	−	−	−	−
1 to 400	+	−	−	−	−	−
1 to 600	+	+	−	+	+	+
1 to 800	+	+	+	+	+	+

EXPERIMENT 3

Concentration of chloramin T.	*B. pyocyaneus.*			*B. typhosus.*		
	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.
1 to 200	+	−	−	−	−	−
1 to 400	+	+	+	−	−	−
1 to 600	+	+	+	+	+	+
1 to 800	+	+	+	+	+	+

[a] + Signifies growth; −, no growth.

EXPERIMENTS 4 AND 5.—These were preliminary experiments with Dakin's solution, which was made up by the use of sodium carbonate alone, as described in the first of the methods of preparation previously mentioned. In order to determine what influence the boric acid exerts, two portions were tested, one with and the other without the addition of boric acid. The technic was the same as that described for experiments 2 and 3. Dilutions given are based on the amount of sodium hypochlorite. The results of these experiments are given in Table III.

The results given in Table III indicate that the boric acid adds somewhat to the germicidal power of Dakin's solution. This is probably due to the small amount of hypochlorous acid set free by the boric acid.

EXPERIMENTS 6, 7, AND 8.—These experiments were made in order to compare the germicidal powers of chloramin T and Dakin's solution against *Staphylococcus aureus*, *Bacillus pyocyaneus*, and *B. typhosus*. The technic was the same as that described for experiments 2 and 3, except for the omission of the 15-minute exposures. The results are given in Table IV.

TABLE III.—*Germicidal efficiency of Dakin's solution against Staphylococcus aureus, with and without boric acid*[a]

Concentration of NaOCl.	Without boric acid.			With boric acid.		
	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.
1 to 100	+	−	−	−	−	−
1 to 200	+	+	−	+	−	−
1 to 400	+	+	−	+	+	−
1 to 800	+	+	+	+	+	+
EXPERIMENT 5						
1 to 100	+	−	−	−	−	−
1 to 200	+	+	−	+	−	−
1 to 400	+	+	+	+	−	−
1 to 600	+	+	+	+	+	+
1 to 800	+	+	+	+	+	+

[a] + signifies growth; −, no growth.

TABLE IV.—*Comparative germicidal efficiency of chloramin T and Dakin's solution against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus, in the presence of 50 per cent blood serum*[a]

EXPERIMENT 6

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.
Chloramin T:						
1 to 200	−	−	−	−	−	−
1 to 300	−	−	+	−	−	−
1 to 400	−	−	+	+	−	−
1 to 500	−	−	+	+	+	−
1 to 600	−	−	+	+	+	+
1 to 800	+	−	+	+	+	+
Dakin's solution:						
NaOCl 1 to 200	−	−	−	−	−	−
NaOCl 1 to 300	+	−	−	−	−	−
NaOCl 1 to 400	+	−	+	−	+	−
NaOCl 1 to 500	+	+	+	−	+	−
NaOCl 1 to 600	+	+	+	−	+	−
NaOCl 1 to 800	+	+	+	+	+	+
EXPERIMENT 7						
Chloramin T:						
1 to 200	No test.	No test.	−	−	−	−
1 to 300	...do....	...do....	−	−	−	−
1 to 400	...do....	...do....	+	−	−	−
1 to 500	+	−	+	+	−	−
1 to 600	+	−	+	+	+	+
1 to 800	+	+	No test.	No test.	+	+
1 to 1,000	+	+	...do....	...do....	No test.	No test.

[a] + signifies growth; −, no growth.

TABLE IV.—*Comparative germicidal efficiency of chloramin T and Dakin's solution against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus, in the presence of 50 per cent blood serum*—Continued

EXPERIMENT 7—continued

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.
Dakin's solution:						
NaOCl 1 to 200.....	+	−	No test.	No test.	No test.	No test.
NaOCl 1 to 300.....	+	−	−	−	−	−
NaOCl 1 to 400.....	+	+	−	−	+	−
NaOCl 1 to 500.....	+	+	+	−	+	−
NaOCl 1 to 600.....	+	+	+	−	+	−
NaOCl 1 to 800.....	No test.	No test.	+	+	+	+
NaOCl 1 to 1,000....	...do....	...do....	+	+	+	+

EXPERIMENT 8

Chloramin T:						
1 to 200.....	No test.	No test.	−	−	−	−
1 to 300.....	...do....	...do....	+	−	−	−
1 to 400.....	−	−	+	+	+	+
1 to 500.....	−	−	+	+	+	+
1 to 600.....	+	−	+	+	+	+
1 to 800.....	+	+	No test.	No test.	No test.	No test.
1 to 1,000.....	+	+	...do....	...do....	...do....	Do.
Dakin's solution:						
NaOCl 1 to 200.....	+	−	...do....	...do....	−	−
NaOCl 1 to 300.....	+	−	−	−	No test.	No test.
NaOCl 1 to 400.....	+	−	+	−	+	−
NaOCl 1 to 500.....	+	−	+	−	No test.	No test.
NaOCl 1 to 600.....	+	+	+	−	+	−
NaOCl 1 to 800.....	+	+	+	+	+	+
NaOCl 1 to 1,000....	No test.	No test.	+	+	+	+

EXPERIMENTS 9, 10, AND 11.—These experiments were undertaken to determine the efficiency of chloramin T and Dakin's solution against *Staphylococcus aureus*, *Bacillus pyocyaneus*, and *B. typhosus* without the addition of blood serum. They are in contrast with the three preceding experiments, in which 50 per cent of blood serum was used.

The technic was as follows: Two drops of a 24-hour bouillon culture were added to 5 cc. of disinfectant, and the mixture was well shaken. After intervals of 1 hour and 2 hours, respectively, the mixtures were again shaken and subcultures were made with a 3-mm. platinum loop into tubes of standard bouillon containing enough sodium thiosulphate to neutralize the disinfectant carried over. The results are given in Table V.

In the results shown in Tables IV and V there is seen evidence of what may be called "selective" action on the part of the two disinfectants tested. For instance, the amount of chloramin T required to kill *Staphylococcus aureus* is very much less than that required to kill *Bacillus pyocyaneus*. In like manner in the presence of blood serum it requires more

Dakin's solution to kill *Staph. aureus* than to kill *B. pyocyaneus* or *B. typhosus* under like conditions. A comparison of the two disinfectants shows that Dakin's solution is more effective than chloramin T against *B. pyocyaneus*, while chloramin T is more effective than sodium hypochlorite against *Staph. aureus*.

TABLE V.—*Comparative germicidal efficiency of chloramin T and Dakin's solution against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus, without addition of blood serum 1*[a]

EXPERIMENT 9

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.
Chloramin T:						
1 to 1,000	−	−	−	−	−	−
1 to 10,000	−	−	+	+	+	−
1 to 25,000	−	−	+	+	+	−
1 to 50,000	+	+	+	+	+	+
1 to 100,000	+	+	+	+	+	+
Dakin's solution:						
NaOCl 1 to 1,000	−	−	−	−	−	−
NaOCl 1 to 10,000	−	−	−	−	−	−
NaOCl 1 to 25,000	−	−	−	−	−	−
NaOCl 1 to 50,000	−	−	−	−	+	+
NaOCl 1 to 100,000	+	+	+	+	+	+

EXPERIMENT 10

Chloramin T:						
1 to 1,000	−	−	−	−	−	−
1 to 10,000	−	−	+	+	+	−
1 to 30,000	−	−	+	+	+	+
1 to 50,000	+	+	+	+	+	+
1 to 100,000	+	+	+	+	+	+
Dakin's solution:						
NaOCl 1 to 1,000	−	−	−	−	−	−
NaOCl 1 to 10,000	−	−	−	−	−	−
NaOCl 1 to 30,000	−	−	+	+	−	−
NaOCl 1 to 50,000	+	−	+	+	+	+
NaOCl 1 to 100,000	+	−	+	+	+	+

EXPERIMENT 11

Chloramin T:						
1 to 1,000	−	−	+	−	−	−
1 to 10,000	−	−	+	+	−	−
1 to 30,000	−	−	+	+	+	−
1 to 50,000	−	−	+	+	+	−
1 to 100,000	+	+	+	+	+	+
Dakin's solution:						
NaOCl 1 to 1,000	−	−	−	−	−	−
NaOCl 1 to 10,000	−	−	−	−	−	−
NaOCl 1 to 30,000	−	−	+	+	−	−
NaOCl 1 to 50,000	−	−	+	+	−	−
NaOCl 1 to 100,000	+	+	+	+	+	+

[a] + signifies growth; −, no growth.

EXPERIMENTS 12 AND 13.—These experiments were made for the purpose of comparing the germicidal activity of chloramin T, Dakin's solution, and eusol against *Staphylococcus aureus*, *Bacillus pyocyaneus*, and *B. typhosus*.

The technic was as follows: Each dilution of the disinfectant was mixed with an equal amount of a 24-hour culture of the test organism, and the mixture was thoroughly shaken. After intervals of 10 minutes and 30 minutes, respectively, the mixtures were again shaken, and subcultures were made with a 3-mm. platinum loop into tubes of standard bouillon containing enough sodium thiosulphate to neutralize the disinfectant carried over. The amounts of culture and disinfectant used were 2.5 cc. of each. For purposes of comparison, tests were made with mercuric chlorid, and in these tests sodium sulphid was used to neutralize the disinfectant carried over. The results are given in Table VI.

TABLE VI.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus* [a]

EXPERIMENT 12

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 10 minutes.	Exposed 30 minutes.	Exposed 10 minutes.	Exposed 30 minutes.	Exposed 10 minutes.	Exposed 30 minutes.
Chloramin T:						
1 to 1,000	−	−	+	−	−	−
1 to 2,000	−	−	+	+	−	−
Dakin's solution:						
NaOCl 1 to 2,000	−	−	−	−	−	−
Eusol:						
HOCl 1 to 2,000	−	−	−	−	−	−
Mercuric chlorid:						
1 to 2,000	−	−	−	−	−	−

EXPERIMENT 13

Chloramin T:						
1 to 1,000	−	−	+	−	−	−
1 to 2,000	−	−	+	+	−	−
Dakin's solution:						
NaOCl 1 to 2,000	−	−	−	−	−	−
NaOCl 1 to 4,000	−	−	−	−	−	−
Eusol:						
HOCl 1 to 2,000	−	−	−	−	−	−
HOCl 1 to 4,000	−	−	−	−	−	−
Mercuric chlorid:						
1 to 2,000	+	+	−	−	+	−
1 to 4,000	+	+	−	−	+	−

[a] + signifies growth; −, no growth.

EXPERIMENTS 14 AND 15.—In these experiments the same disinfectants were compared as in experiments 12 and 13, but with the addition of blood serum. The technic was the same also, except that a mixture of equal parts of blood serum and culture was used instead of culture alone. The results are given in Table VII.

TABLE VII.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus in the presence of 25 per cent blood serum*[a]

EXPERIMENT 14

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 10 minutes	Exposed 30 minutes	Exposed 10 minutes.	Exposed 30 minutes.	Exposed 10 minutes.	Exposed 30 minutes.
Chloramin T:						
1 to 500	−	−	+	−	−	−
1 to 1,000	−	−	+	+	+	+
Dakin's solution:						
NaOCl 1 to 2,000	−	−	−	−	−	−
Eusol:						
HOCl 1 to 2,000	−	−	−	−	−	−
Mercuric chlorid:						
1 to 2,000	+	−	+	−	+	−

EXPERIMENT 15

Chloramin T:						
1 to 1,000	−	−	+	+	+	+
1 to 2,000	+	−	+	+	+	+
Dakin's solution:						
NaOCl 1 to 2,000	+	−	−	−	+	−
NaOCl 1 to 4,000	+	−	+	+	+	−
Eusol:						
HOCl 1 to 2,000	−	−	−	−	−	−
HOCl 1 to 4,000	−	−	−	−	−	−
Mercuric chlorid:						
1 to 2,000	+	+	−	−	+	−
1 to 4,000	+	+	−	−	+	−

[a] + signifies growth; − no growth.

The four experiments shown in Tables VI and VII show that eusol is decidedly superior to chloramin T, Dakin's solution, and mercuric chlorid, especially in the presence of blood serum. Unfortunately, however, eusol is very unstable and for that reason is not reliable, since it is impossible in practice to count on its containing any uniform amount of active material. In the tests reported here the eusol was made up just before the test and was used as soon as possible, but observations which were made in connection with chemical work upon these various disinfectants would tend to show that there was probably a perceptible loss of strength even in the time required for a test.

EXPERIMENTS WITH ANTHRAX SPORES

The experiments upon anthrax spores were performed by the Hill (6) "rod" method, with some modifications. The method as modified is as follows: Glass rods $^3/_{16}$-inch in diameter and 8 inches long are etched at one end, the etched portion being about 1 inch long. Cotton is wrapped

about the rods near the end not etched, and the rods are thrust into test tubes so as to engage the cotton in the mouth of the tube. The tubes containing the rods are sterilized by dry heat (150° C.) for 1 hour or more. In making tests the rods are removed from the tubes and the etched portions are dipped into a suspension made from a culture of the organism to be tested. They are then replaced in the tubes and dried in the incubator for one hour.

Rods so infected are transferred to test tubes containing the disinfectant to be tested, the amount of disinfectant being sufficient to cover all the infected portion of the rod. They are exposed to the action of the disinfectant for varying lengths of time. After exposure the rods are washed with sterile water in order to remove traces of the disinfectant and are then transferred to tubes containing bouillon or agar, which are incubated for at least 48 hours at 37.5° C. The suspension used in infecting the rods is made from the surface growth on an agar tube by rubbing up in several cubic centimeters of sterile water enough of the growth to give a suspension of approximately the same density as a 24-hour bouillon culture of *Bacillus typhosus*. For an organism that does not bear spores the culture should be 24 hours old, while for spore-bearing organisms cultures 1 to 2 weeks old are usually the most suitable.

In making tests with a disinfectant containing mercury it is advisable to dip the rods into a saturated solution of hydrogen sulphid or an aqueous solution of some sulphid before placing them in subculture tubes. In this connection it should be mentioned that media of acid reaction have been found to exert an inhibitory action upon the growth of *Bacillus anthracis* after exposure to disinfectants. For that reason the media used in these experiments have been neutral or slightly alkaline.

EXPERIMENTS 16 AND 17.—In these experiments chloramin T was tested in varying concentrations, both in water and in 50 per cent blood serum. A sterile 10 per cent solution of sodium thiosulphate was used for washing the rods before placing them in subculture tubes of exactly neutral broth. The results are given in Table VIII.

TABLE VIII.—*Germicidal efficiency of chloramin T against anthrax spores, with and without the addition of blood serum* [a]

EXPERIMENT 16

Concentration of chloramin T.	Amount of serum.	Exposed 2 hours.	Exposed 4 hours.	Exposed 24 hours.
Per cent.				
10	None	+	+	−
10	50 per cent	+	−	−
5	None	+	+	−
5	50 per cent	−	−	−
1	None	+	+	−
1	50 per cent	+	+	−
Control rod			+	+

[a] + signifies growth; −, no growth.

TABLE VIII.—*Germicidal efficiency of chloramin T against anthrax spores, with and without the addition of blood serum*—Continued.

EXPERIMENT 17

Concentration of chloramin T.	Amount of serum.	Exposed 2 hours.	Exposed 4 hours.	Exposed 24 hours.
Per cent.				
5	None	+	+	−
5	50 per cent	+	−	−
1	None	+	+	−
1	50 per cent	+	+	−
0.5	None	+	+	+
0.5	50 per cent	+	+	+
Control rod			+	+

EXPERIMENT 18.—This experiment was undertaken in order to compare chloramin T and Dakin's solution. The results are given in Table IX.

TABLE IX.—*Comparative germicidal efficiency of chloramin T and Dakin's solution against anthrax spores, with and without the addition of blood serum* [a]

EXPERIMENT 18

Disinfectant and dilution.	Amount of serum.	Exposed 2 hours.	Exposed 4 hours.	Exposed 24 hours.
Chloramin T:				
2 per cent	None	+	+	+
Do	50 per cent	+	+	−
1 per cent	None	+	+	+
Do	50 per cent	+	+	−
0.5 per cent	None	+	+	+
Do	50 per cent	+	+	+
Dakin's solution:				
NaOCl 2 per cent	None	−	−	−
Do	50 per cent	+	+	−
NaOCl 1 per cent	None	−	−	−
Do	50 per cent	+	+	−
NaOCl 0.5 per cent	None	−	−	−
Do	50 per cent	+	+	+
Control rod		+	+	+

[a] + signifies growth; −, no growth

EXPERIMENT 19.—In this experiment eusol was used, the chlorin being estimated as HOCl. In the dilutions there was approximately 0.128 gm. HOCl per 100 cc. The results are given in Table X.

TABLE X.—*Germicidal efficiency of eusol against anthrax spores* [a]

EXPERIMENT 19

Time of exposure.	No serum.	50 per cent serum.
30 minutes	+	+
1 hour	+	+
2 hours	+	+
3 hours	+	+
4 hours	−	+
5 hours	−	+
24 hours	−	+

[a] + signifies growth; −, no growth.

EXPERIMENTS 20 AND 21.—In these experiments comparison was made between chloramin T, Dakin's solution, eusol, and mercuric chlorid. The results are given below in Table XI. The results with mercuric chlorid are included for comparison.

TABLE XI.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against anthrax spores, with and without blood serum* [a]

EXPERIMENT 20

Disinfectant and dilution.	Amount of serum.	Exposed 2 hours.	Exposed 4 hours.	Exposed 1 day.	Exposed 2 days.	Exposed 4 days.
Chloramin T:						
1 to 100	None	+	+	+	−	−
1 to 100	50 per cent	+	+	+	+	+
1 to 200	None	+	+	+	−	−
1 to 200	50 per cent	+	+	+	+	+
Dakin's solution:						
NaOCl 1 to 100	None	−	−	−	−	−
NaOCl 1 to 100	50 per cent	+	+	+	+	+
NaOCl 1 to 200	None	−	−	−	−	−
NaOCl 1 to 200	50 per cent	+	+	+	+	+
Eusol:						
HOCl 1 to 200	None	−	−	−	−	−
HOCl 1 to 200	50 per cent	+	+	+	−	−
HOCl 1 to 400	None	−	−	−	−	−
HOCl 1 to 400	50 per cent	+	+	+	+	+
Mercuric chlorid:						
1 to 2,000	None	+	−	−	−	−
1 to 2,000	50 per cent	+	+	+	+	+

EXPERIMENT 21

Chloramin T:						
5 per cent	25 per cent	.....	+	−	−	−
5 per cent	50 per cent	.....	+	−	−	−
5 per cent	None	+	+	+	−	−
Dakin's solution:						
NaOCl 1 per cent	25 per cent	.....	+	+	+	.....
NaOCl 1 per cent	50 per cent	.....	.....	+	+	+
NaOCl 1 per cent	None	−	−	−	−	−
Eusol:						
HOCl 0.75 per cent	25 per cent	.....	−	−	−	.....
HOCl 0.75 per cent	50 per cent	.....	.....	+	−	−
HOCl 0.75 per cent	None	−	−	−	.....	.....

+[a] signifies growth; −, no growth.

It should be noted here that in the experiments upon anthrax spores the strength of NaOCl and HOCl required was in most instances greater than that obtained by preparing Dakin's solution and eusol by the methods described at the beginning of this paper. So in these instances the solutions were made with less water in proportion to the other ingredients. Aside from this change the methods of preparation were the same.

The experiments upon anthrax spores indicate that if comparison is made on the basis of weight of chloramin T against weight of chlorin

as NaOCl or HOCl, in Dakin's solution and eusol, respectively, chloramin T must be regarded as less efficient than Dakin's solution or eusol against naked spores. In the presence of blood serum it is more or less equal to Dakin's solution, while eusol seems to be superior to both chloramin T and Dakin's solution. Comparison on the basis of "available" chlorin would, of course, be much more favorable to chloramin, since it contains only 25 per cent available chlorin, or 12½ per cent actual chlorin.

It is interesting to note that in experiments 16, 17, 18, and 21 chloramin T was more efficient against anthrax spores in the presence of blood serum than in the absence of serum. In experiments 16 and 17 this is true only for the stronger dilutions (10 per cent and 5 per cent) and is not true for the lowest dilution (1 per cent). In experiment 18 it is true for 2 per cent and 1 per cent dilutions after 24 hours, but in experiment 20 with dilutions of 1 to 100 and 1 to 200 and exposures of 2 days there is greater efficiency without serum than with it. Experiment 21 confirms the results obtained in experiments 16 and 17 with a 5 per cent dilution.

These experiments also seem more or less at variance with the widely expressed opinion that chlorin compounds rapidly lose their activity and soon become inert, especially in the presence of organic matter. For example, in experiment 20, HOCl 1 to 200 did not destroy anthrax spores until after an exposure of 2 days, the 4-day result serving as a control to show the correctness of the result.

This usually accepted opinion is controverted by Rideal (*9*), who as the result of his own experiments concludes that—

> chlorin has a disinfectant value out of all proportion to that which would be expected from the hitherto accepted theories, even in the presence of a chemical excess of organic matter in certain forms.

The explanation which he gives is that the disinfecting action of chlorin is not due merely to oxidation but also to the action of products formed by its substitution for hydrogen in ammonia and organic compounds.

EXPERIMENTS WITH BACILLUS TUBERCULOSIS

In experiments upon the tubercle bacillus the method was as follows Two and one-half cc. of disinfectant dilution were added to 2½ cc. of a suspension of culture (or a mixture of such suspension with an equal quantity of horse-blood serum), and they were mixed thoroughly by vigorous shaking. The suspension was made by rubbing up in sterile distilled water enough of the surface growth from a bouillon culture to give a suspension whose density was approximately equal to that of a 24-hour culture of *Bacillus typhosus*. After an exposure of 10 minutes enough sterile sodium thiosulphate solution (or sodium sulphid where mercuric chlorid was used) was added to insure complete neutralization, and finally 1 cc. of each neutralized test mixture was injected subcutaneously into a guinea pig.

This technic was used in making a number of comparative experiments with chloramin T, Dakin's solution, eusol, and mercuric chlorid. The results are given in Tables XII and XIII.

TABLE XII.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against Bacillus tuberculosis, with and without the addition of 25 per cent blood serum*

EXPERIMENT 22

Disinfectant and dilution.	Amount of serum.	Guinea pig No.	Result.	Autopsy.
Chloramin T:				
1 to 1,000	None	53414	Died	Typical lesions.
Do	25 per cent	53415	do	Do.
Dakin's solution:				
NaOCl 1 to 1,000	None	53416	do	Do.
Do	25 per cent	53417	do	Do.
Eusol:				
HOCl 1 to 1,000	None	53418	Killed[a]	
Do	25 per cent	53419	Died	Do.
Mercuric chlorid:				
1 to 1,000	None	53420	do	Do.
Do	25 per cent	53421	do	Do.
Tubercle bacillus suspension.		53422	do	Do.
Do	+ serum	53423	do	Do.

EXPERIMENT 23

Chloramin T:				
1 to 100	None	54023	Died	Typical lesions.
Do	25 per cent	54024	do	Do.
Dakin's solution:				
NaOCl 1 to 500	None	54025	do	Do.
Do	25 per cent	54026	do	Do.
Eusol:				
HOCl 1 to 250	do	54027	do	Do.
HOCl 1 to 500	do	54028	do	Do.
Mercuric chlorid:				
1 to 500	None	54031	Killed[b]	
Do	25 per cent	54032	Died	Do.
Tubercle bacillus suspension.		54033	do	Do.
Do	+serum	54034	do	Do.

[a] Killed after 33 days; perfectly normal. [b] Killed after 2 months; perfectly normal.

EXPERIMENTS 24 AND 25.—In these experiments the disinfectants were compared on the basis of the available chlorin contained, so these experiments are grouped by themselves in Table XIII.

In the experiments upon *Bacillus tuberculosis*, as in experiments upon anthrax spores, it was necessary to use Dakin's solution and eusol of greater strength, and, as before, this result was obtained by lessening the amount of water while the other ingredients and the method of manufacture remained unchanged.

TABLE XIII.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against Bacillus tuberculosis, with dilutions based on available chlorin*

EXPERIMENT 24

Disinfectant and dilution.	Available chlorin.	Amount of serum.	Guinea pig No.	Result.	Autopsy.
Chloramin T.....	1 to 200	None.	54549	Died...	Typical lesions.
Do.....	1 to 200	25 per cent.	54550	...do....	Do.
Eusol.....	1 to 200	None.	54551	Killed[a]	Do.
Do.....	1 to 200	25 per cent.	54552	Died...	Do.
Dakin's solution.....	1 to 200	None.	54555	...do....	Not tuberculous.[b]
Do.....	1 to 200	25 per cent.	54556	...do....	Typical lesions.
Mercuric chlorid:					
1 to 500.....	.....	None.	54557	Killed[a]	Normal.
Do.....	.....	25 per cent.	54558	Died...	Typical lesions.
Tubercle bacillus suspension.	.....	None.	54559	...do....	Do.
Do.....	.....	25 per cent.	54560	...do....	Do.

EXPERIMENT 25

Chloramin T.....	1 to 200	None.	55552	Died...	Typical lesions.
Do.....	1 to 200	25 per cent.	55553	...do....	Do.
Eusol.....	1 to 300	None.	55554	...do....	Not tuberculous.[c]
Do.....	1 to 300	25 per cent.	55555	...do....	Typical lesions.
Dakin's solution.....	1 to 200	None.	55558	...do....	Not tuberculous.[d]
Do.....	1 to 200	25 per cent.	55559	...do....	Typical lesions.
Mercuric chlorid:					
1 to 500.....	.....	None.	55560	Killed[e]	Normal.
Do.....	.....	25 per cent.	55561	Died...	Typical lesions.
Tubercle bacillus suspension.	.....	None.	55562	...do....	Do.
Do.....	.....	25 per cent.	55563	...do....	Do.

[a] Killed after 10 weeks.
[b] Died after 7 weeks of an intercurrent pneumonia.
[c] Died after 2 months; no lesions observed; death probably due to scurvy.
[d] Died after 1 month of an intercurrent pneumonia.
[e] Killed after 2 months; perfectly normal.

The results of the experiments upon the tubercle bacillus would seem to indicate that the chlorin compounds are entirely inefficient so far as that organism is concerned. These are the results to be expected in view of the use of antiformin for isolating tubercle bacilli.[1]

CARBOLIC-ACID COEFFICIENTS OF THE CHLORIN ANTISEPTICS

The results here given are those of a large number of tests made by the Rideal-Walker method (*10*), modified only as stated below. Aside from the use of *Staphylococcus aureus* and *Bacillus pyocyaneus* as test organisms in addition to *B. typhosus*, the only modifications were the use of bacto-peptone instead of Witte's peptone and a relaxation of the rule that coefficients are to be deduced only where there is life after 5 minutes and death after 7½ minutes.

On account of variation in the resistance of the cultures, especially *Staphylococcus aureus* and *Bacillus pyocyaneus*, it was inconvenient to

[1] Amounts actually found inefficient were as follows: Chloramin T, 1 to 50; eusol, 0.5 per cent; and Dakin's solution, 0.5 per cent.

adhere strictly to the rule; and coefficients were deduced at any time within the 15-minute period, except that no coefficient was deduced unless there was growth in the phenol subculture tubes after both 2½ and 5 minutes' exposure. This is really only a return to previous practice (7), and the results obtained are sufficiently accurate for all practical purposes.

In all these tests, dilutions were based on the amount of available chlorin; and it should, therefore, be understood that the coefficients are really, so to speak, those of available chlorin as it is present in chloramin T, eusol, Dakin's solution, and chlorin water.

It should also be noted that in order to make the original solutions more nearly equal in chlorin content the amount of bleaching powder in proportion to water was the same for eusol as for Dakin's solution. The amount used was 5 gm. to 250 cc., which follows the usual proportion for Dakin's solution but varies from the usual proportion for eusol. These original solutions were then diluted with distilled water to obtain the desired amounts of available chlorin in the various dilutions.

The results are summarized in Table XIV, the successive figures from top to bottom in each column being coefficients obtained at various times. It will be noted that they do not always agree perfectly, but they are not offered as examples of accuracy. On the contrary, they are to be considered as approximate values to be taken for what they are worth as illustrations of the general principles of selective action already shown to a greater or less degree in previous experiments.

TABLE XIV.—*Coefficients of chloramin T, Dakin's solution, eusol, and chlorin water, based on the content of available chlorin*

Chloramin T.			Dakin's solution.			Eusol.			Chlorin water.		
Staphylococcus aureus.	*Bacillus pyocyaneus.*	*Bacillus typhosus.*	*Staphylococcus aureus.*	*Bacillus pyocyaneus.*	*Bacillus typhosus.*	*Staphylococcus aureus.*	*Bacillus pyocyaneus.*	*Bacillus typhosus.*	*Staphylococcus aureus.*	*Bacillus pyocyaneus.*	*Bacillus typhosus.*
114	8. 3	66. 6	57	66. 6	66. 6	114	120	100	92. 3	80	88
92. 3	8. 8	66. 6	57	55. 5	66. 6	92. 3	120	111	92. 3	80	80
92. 3	8. 3	66. 6	57	66. 6	66. 6	92. 3	120	111	92. 3	80	80
92. 3	.....	.....	.....	66. 6	.....	92. 3	.....	111	.....	.....	.....
92. 3	.....	.....	.....	55. 5	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	66. 6	.....	.....	.....	.....	.....	.....	.....

In connection with preceding experiments solutions of chlorin T, Dakin's solution, and eusol were kept in a dark closet at room temperature, and titrations were made at intervals to detect any changes that might occur. It was found that Dakin's solution and solutions of chloramin T will keep for a month or more without any great loss of available chlorin; while, on the other hand, eusol deteriorates rapidly, there being a noticeable change even within 24 hours. For example, in one instance a sample of Dakin's solution showed only about 10 per cent loss after standing 6

months, while a sample of eusol lost 10 per cent of its available chlorin in 24 hours.

In view of the instability of eusol an attempt was made to secure a more stable product by reducing the amount of boric acid, and it was found that by reducing the proportions from equal parts of bleaching powder and boric acid to 10 parts of bleaching powder and 3 parts of boric acid a product was obtained which was fully as stable as Dakin's solution. This modified eusol was tested by the carbolic-acid coefficient method in comparison with the regular eusol. The results are given in Table XV. Eusol made by the original formula is designated as eusol I, while that made by the modified formula is designated as eusol II.

TABLE XV.—*Carbolic-acid coefficients of eusol I (original formula) and eusol II (modified formula), based on available chlorin*

Date.	Solution.	Coefficient with *Staphylococcus aureus.*	Coefficient with *Bacillus pyocyaneus.*	Coefficient with *Bacillus typhosus.*
Feb. 4	Eusol I	92.3	120	111
Do	Eusol II	77	80	66.6
Feb. 7	Eusol I	92.3	120	100
Do	Eusol II	77	80	66.6

According to the results of these comparative tests it would seem that eusol I is superior to eusol II in germicidal efficiency.

INFLUENCE OF AMMONIA UPON THE GERMICIDAL EFFICIENCY OF CHLORIN DISINFECTANTS

It has been shown by Race (*8*) and Rideal (*9*) that the addition of ammonia to electrolytic hypochlorite solutions greatly increases their germicidal efficiency. Their explanation of this increase is that it is due to the formation of chloramin (NH_2Cl). The experiments here discussed were intended to verify these findings by the use of methods similar to those employed in the experiments already discussed, without attempting to ascertain the cause of the increased germicidal value.

The method first used was the Rideal-Walker method (*10*), modified by the use of an unadjusted culture medium as recommended by the American Public Health Association Committee on Standard Methods of Examining Disinfectants (*11*). The method was also modified by deducing coefficients at times other than 7½ minutes, and in many instances no coefficient was obtained.

By the use of this method experiments were first made upon Dakin's solution, prepared from bleaching powder by the use of sodium carbonate and bicarbonate as previously described. Ammonia was added so as to furnish a molecular equivalent to the sodium hypochlorite of the Dakin's solution. Experiment 26 (Table XVI) shows the comparative results with no organic matter added, and experiments 27 and 28 (Table XVI) show the results with blood serum added.

TABLE XVI.—*Effect of addition of ammonia upon germicidal activity of Dakin's solution against Bacillus typhosus*[a]

EXPERIMENT 26

WITHOUT AMMONIA; NO BLOOD SERUM ADDED

Concentration of NaOCl.	Exposed 2½ minutes.	Exposed 5 minutes.	Exposed 7½ minutes.	Exposed 10 minutes.	Exposed 12½ minutes.	Exposed 15 minutes.	
1 to 2,000	−	−	−	−	−	−	
1 to 4,000	+	+	+	−	−	−	Coefficient 57,
1 to 6,000	+	+	+	+	+	+	$\frac{4,000}{70} = 57$.
1 to 8,000	+	+	+	+	+	+	
Phenol 1 to 70	+	+	+	−	−	−	

WITH AMMONIA; NO BLOOD SERUM ADDED

1 to 6,000	+	+	−	−	−	−	
1 to 8,000	+	+	+	−	−	−	Coefficient 86,
1 to 10,000	+	+	+	−	−	−	$\frac{6,000}{70} = 86$.
1 to 12,000	+	+	+	+	−	−	
Phenol 1 to 70	+	+	−	−	−	−	

EXPERIMENT 27

WITHOUT AMMONIA; 5 PER CENT BLOOD SERUM ADDED

1 to 500	+	+	+	+	+	+	
1 to 1,000	+	+	+	+	+	+	
1 to 2,000	+	+	+	+	+	+	
1 to 4,000	+	+	+	+	+	+	
Phenol 1 to 80[b]	+	+	+	+	+	−	

WITH AMMONIA; 5 PER CENT BLOOD SERUM ADDED

1 to 500	−	−	−	−	−	−	
1 to 1,000	−	−	−	−	−	−	
1 to 2,000	−	−	−	−	−	−	
1 to 4,000	+	−	−	−	−	−	
Phenol 1 to 80[b]	+	+	+	+	−	−	

EXPERIMENT 28

WITH AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 500	−	−	−	−	−	−	
1 to 1,000	+	−	−	−	−	−	
1 to 2,000	+	+	+	−	−	−	
1 to 4,000	+	+	+	+	+	+	
Phenol 1 to 70[b]	+	−	−	−	−	−	

WITH AMMONIA; 50 PER CENT BLOOD SERUM ADDED

1 to 500	+	+	+	+	+	−	
1 to 1,000	+	+	+	+	+	+	
1 to 1,500	+	+	+	+	+	+	
1 to 2,000	+	+	+	+	+	+	
Phenol 1 to 70[b]	+	−	−	−	−	−	

[a] + signifies growth; −, no growth.

[b] No blood serum added.

The experiments shown above in Table XVI indicate that the addition of a molecular equivalent of ammonia to Dakin's solution not only greatly increases its germicidal value against "naked" bacteria, but, to a large extent, prevents depreciation of germicidal value due to the addition of blood serum.

In Table XVII there are shown the results of a number of experiments upon chlorin water, with and without a molecular equivalent of ammonia.

TABLE XVII.—*Effect of ammonia upon the germicidal activity of chlorin in aqueous solution against Bacillus typhosus* [a]

EXPERIMENT 29

WITHOUT AMMONIA

Concentration of chlorin.	Exposed 2½ minutes.	Exposed 5 minutes.	Exposed 7½ minutes.	Exposed 10 minutes.	Exposed 12½ minutes.	Exposed 15 minutes.
1 to 4,000	−	−	−	−	−	−
1 to 8,000	−	−	−	−	−	−
1 to 12,000	−	−	−	−	−	−
1 to 16,000	+	+	−	−	−	−
Phenol 1 to 80	+	+	+	−	−	−

WITH AMMONIA

1 to 4,000	−	−	−	−	−	−
1 to 8,000	+	−	−	−	−	−
1 to 12,000	+	+	+	−	−	−
1 to 16,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	+	−	−	−

EXPERIMENT 30

WITHOUT AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 500	−	−	−	−	−	−
1 to 1,000	+	−	−	−	−	−
1 to 2,000	+	+	+	+	+	+
1 to 4,000	+	+	+	+	+	+
Phenol 1 to 80 [b]	+	+	+	+	+	−

WITH AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 1,000	−	−	−	−	−	−
1 to 2,000	−	−	−	−	−	−
1 to 4,000	−	−	−	−	−	−
1 to 6,000	−	−	−	−	−	−
Phenol 1 to 80 [b]	−	−	−	−	−	−

[a] + signifies growth; −, no growth.

[b] No blood serum added.

The experiments shown in Table XVII indicate that the addition of a molecular equivalent of ammonia to chlorin water decreases rather than increases the germicidal value of the chlorin in the absence of organic matter, but it does tend to prevent depreciation of germicidal activity on the addition of blood serum.

The experiments shown in Table XVIII were designed to determine the optimum amount of ammonia.

TABLE XVIII.—*Effect of varying amounts of ammonia upon the germicidal value of chlorin in aqueous solution* [a]

EXPERIMENT 31

WITH MOLECULAR EQUIVALENT OF AMMONIA

Concentration of chlorin.	Exposed 2½ minutes.	Exposed 5 minutes.	Exposed 7½ minutes.	Exposed 10 minutes.	Exposed 12½ minutes	Exposed 15 minutes.
1 to 5,000	−	−	−	−	−	−
1 to 10,000	+	+	−	−	−	−
1 to 15,000	+	+	+	+	+	+
1 to 20,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	+	−	−	−

WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA

1 to 5,000	−	−	−	−	−	−
1 to 10,000	−	−	−	−	−	−
1 to 15,000	−	−	−	−	−	−
1 to 20,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	+	+	−	−

EXPERIMENT 32

WITH MOLECULAR EQUIVALENT OF AMMONIA

1 to 5,000	−	−	−	−	−	−
1 to 10,000	+	−	−	−	−	−
1 to 15,000	+	+	+	+	−	−
1 to 20,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

WITH TWO MOLECULAR EQUIVALENTS OF AMMONIA

1 to 5,000	+	+	+	+	−	−
1 to 10,000	+	+	+	+	+	+
1 to 15,000	+	+	+	+	+	+
1 to 20,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

[a] + signifies growth −, no growth.

TABLE XVIII.—*Effect of varying amounts of ammonia upon the germicidal value of chlorin in aqueous solution*—Continued

EXPERIMENT 33

WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA

Concentration of chlorin.	Exposed 2½ minutes.	Exposed 5 minutes.	Exposed 7½ minutes.	Exposed 10 minutes.	Exposed 12½ minutes.	Exposed 15 minutes.
1 to 10,000	−	−	−	−	−	−
1 to 15,000	+	+	+	+	+	−
1 to 20,000	+	+	+	+	+	+
1 to 25,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

WITH ONE-FOURTH MOLECULAR EQUIVALENT OF AMMONIA

1 to 10,000	−	−	−	−	−	−
1 to 15,000	+	+	+	+	+	−
1 to 20,000	+	+	+	+	+	+
1 to 25,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

EXPERIMENT 34

WITH MOLECULAR EQUIVALENT OF AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 1,000	+	+	+	+	−	−
1 to 2,000	+	+	+	+	+	+
1 to 4,000	+	+	+	+	+	+
1 to 6,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 1,000	+	−	−	−	−	−
1 to 2,000	+	+	+	+	+	+
1 to 4,000	+	+	+	+	+	+
1 to 6,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

The experiments shown in Table XVIII indicate that the optimum amount of ammonia is approximately one-half of the molecular equivalent.

Experiments were next made with anthrax spores, using the following method: Equal quantities (2½ cc. each) of chlorin solution and spore suspension, with or without blood serum added to it, were mixed in a test tube and vigorously shaken. After it had stood at room temperature for the required time of exposure the mixture was again shaken, and a subculture was made by a standard platinum loop into a tube of nutrient broth. No attempt was made to neutralize any excess of disinfectant. The results of these experiments are shown in Table XIX.

TABLE XIX.—*Germicidal activity of chlorin against anthrax spores with and without addition of ammonia* [a]

EXPERIMENT 35

WITHOUT AMMONIA; 10 PER CENT BLOOD SERUM ADDED

Concentration of chlorin.	Exposed 1 hour.	Exposed 2 hours.	Exposed 3 hours.	Exposed 4 hours.	Exposed 5 hours.	Remarks.
1 to 1,000	+	+	+	+	−	The same dilutions without blood serum killed the spores in 30 minutes.
1 to 2,000	+	+	+	+	+	
1 to 4,000	+	+	+	+	+	
WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA; 10 PER CENT BLOOD SERUM ADDED						
1 to 1,000	−	−	−	−	−	Number of spores 350,000, or 70,000 per cubic centimeter.
1 to 2,000	−	−	−	−	−	
1 to 4,000	+	+	−	+	−	

EXPERIMENT 36

WITHOUT AMMONIA; 10 PER CENT BLOOD SERUM ADDED

Concentration of chlorin.	Exposed 3 hours.	Exposed 6 hours.	Exposed 12 hours.	Exposed 18 hours.	Exposed 24 hours.	Remarks.
1 to 1,000	+	+	+	+	−	The same dilutions without blood serum killed spores in 15 minutes.
1 to 2,000	+	+	+	+	+	
1 to 3,000	+	+	+	+	+	
1 to 4,000	+	+	+	+	+	
WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA; 10 PER CENT BLOOD SERUM ADDED						
1 to 2,000	−	−	−	−	−	Number of spores 350,000, or 70,000 per cubic centimeter.
1 to 4,000	−	−	−	−	−	
1 to 6,000	−	−	−	−	−	
1 to 8,000	+	+	+	+	+	

[a] + signifies growth; −, no growth.

The results shown in Table XIX seemed to show clearly that chlorin with ammonia added had very great germicidal value, even in the presence of organic matter in the form of blood serum.

Experiments were, therefore, undertaken to ascertain whether or not such a solution could be used for disinfecting hides. The technic was as follows: Small pieces of dry hide, cut to the same weight, were infected by soaking them in a suspension of anthrax spores and then drying them over sulphuric acid in a vacuum equal to about 5 mm. of mercury for 48 hours. These pieces of infected hide were then treated with the disinfectant solution in the proportion of 5 times as much solution as hide by weight. At the end of the required period of exposure the pieces of hide were transferred to a solution of sodium thiosulphate of sufficient strength to neutralize completely the disinfectant carried over by the hide. After neutralization the hair and more or

less of the hide surface were scraped off with sterile instruments and plated, using exactly neutral agar. The results of two such experiments are given in Table XX.

It should be noted in this connection that the stock solutions of chlorin water and Dakin's solution from which test dilutions were prepared by the machine devised by the Wallace & Tiernan Co., of New York, for the preparation of Dakin's solution, chlorin being run directly into water or a solution of sodium carbonate, as the case might be.

TABLE XX.—*Germicidal activity of chlorin water and Dakin's solution against anthrax spores on pieces of hide*

EXPERIMENT 37

CHLORIN WATER WITH MOLECULAR EQUIVALENT OF AMMONIA

Concentration of chlorin.	Exposed 2 hours.	Exposed 6 hours.	Exposed 12 hours.	Exposed 24 hours.
1 to 500...	Plates overgrown.	Colonies too many to count.	16 colonies, 14 anthrax.	Many anthrax colonies.
1 to 1,000.	.....do.....	.....do.....	Many anthrax colonies.	Do.
1 to 2,000.	.....do.....	.....do.....	Plate overgrown.	Do.[a]

CHLORIN WATER WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA

1 to 500...	Plates overgrown.	Colonies too many to count.	80 colonies, 70 anthrax.	20 anthrax colonies.
1 to 1,000.	.....do.....	.....do.....	Plates overgrown.	40 anthrax colonies.
1 to 2,000.	.....do.....	.....do.....	.....do.....	Colonies too many to count.

[a] No count possible on account of spreaders.

EXPERIMENT 38

DAKIN'S SOLUTION WITH NO AMMONIA ADDED

Concentration of available chlorin.	Exposed 6 hours.	Exposed 18 hours.	Exposed 24 hours.	Exposed 48 hours.
1 to 250...	Plates all show heavy growth, spreaders plus discrete and confluent colonies of anthrax.			
1 to 500...				

DAKIN'S SOLUTION WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA

1 to 250...	1 colony[a].....	10 colonies[a].....	1 colony[a].....	4 colonies.[a]
1 to 500...	Many anthrax colonies.[b]	4 colonies, 2 anthrax.	No colonies visible.[c]	2 colonies.[a]

DAKIN'S SOLUTION WITH MOLECULAR EQUIVALENT OF AMMONIA

1 to 250...	Many anthrax colonies.[b]	4 colonies.....	1 colony[a].....	4 colonies.[a]
1 to 500...	.....do.....	1 colony anthrax.	No colonies visible.[c]	1 colony.[a]

[a] Not anthrax. [b] No count possible on account of spreaders. [c] Covered by spreaders.

The results shown in Table XX indicate that the results previously obtained were too high, presumably because in the previous experiments there was no attempt to neutralize the disinfectant. However, on the addition of ammonia there is still evident a great increase in germicidal activity and much less decrease in germicidal power when organic matter is present.

A selective action upon the different types of organisms was also seen. Where the concentration of available chlorin was low and the time of exposure comparatively short the plates were overgrown by spreaders and various types of colonies, of which the anthrax colonies made a very small part. With greater concentration of chlorin and longer exposure this proportion was reversed, and most of the colonies seen were those of anthrax. In experiment 38 it was found that even after no anthrax colonies were to be found there were still spreaders and colonies of organisms other than anthrax. These organisms were not identified except to make sure they were not anthrax but were evidently already present on the hide and were more resistant than the strain of anthrax spores employed.

CONCLUSION

(1) In the ordinary routine work of general disinfection, such as disinfection of cattle cars and pens, there is always a large amount of organic matter present. It is evident, therefore, that because of the enormous diminution in germicidal value on addition of organic matter as well as because of the injurious effects on metals and fabrics the chlorin disinfectants as a class do not seem to be suited for use under the usual conditions and by the usual methods of general disinfection. That is not to say, however, that when properly used they are not efficient and valuable in the treatment of infected wounds; in fact, the evidence available goes to show that they are of great value when so used; and, of course, chlorin and hypochlorites are being very widely and successfully used for the disinfection of drinking water.

(2) Compared on a basis of weight of chloramin T as against weight of chlorin as sodium hypochlorite (Dakin's solution) or hypochlorous acid (eusol), or as chlorin in aqueous solution, chloramin T is less efficient than the others. But if the comparison is made on the basis of available chlorin contained it is much more efficient against *Staphylococcus aureus*, much less efficient against *Bacillus pyocyaneus*, and approximately equal in efficiency against *B. typhosus*.

(3) The experiments upon *Bacillus tuberculosis* indicate that the chlorin disinfectants are worth very little so far as that organism is concerned. This is not surprising in view of the use of antiformin (NaOCl + NaOH) in isolating tubercle bacilli.

(4) In the present work, considered as a whole, there is seen throughout more or less "selective action" on the part of the various disinfectants. The most clearly defined example of this is seen in the extremely high

value of chloramin T against *Staphylococcus aureus* as compared with its extremely low value against *Bacillus pyocyaneus*.

(5) The results of the experiments upon anthrax spores show that the germicidal action of chlorin compounds is not always so speedy as is commonly supposed but may extend over several days.

(6) The addition of ammonia to solutions of chlorin or hypochlorites very greatly increases germicidal activity and tends to prevent depreciation in value on the addition of organic matter.

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A NEW AVOCADO WEEVIL FROM THE CANAL ZONE

By H. F. DIETZ,[1] *Entomological Inspector*, with description of the species by H. S. BARBER, *Assistant, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

The Federal quarantine against the avocado weevil (*Heilipus lauri* Boheman) led Mr. James Zetek, Entomologist of the Panama Canal, and the writer, during the service of the latter in the Canal Zone, to search for the weevil in the native avocados in Panama. The weevil proves to be a species previously unknown to science, but the results of investigations of the breeding habits of these potential pests, still very imperfectly understood, supply the first records of field observations under natural conditions.

Two closely related species of avocado weevils are known.[2] As the first, *H. lauri* Boheman, is indigenous to Mexico and the second, *H. pittieri* Barber, is native in Costa Rica, the existence of this new form had already been suspected.[3] Its discovery is of special interest, however, since it has been recently intercepted entering the United States.[4]

FIELD OBSERVATIONS

Two males of this weevil had been found in June, 1918, feeding on the leaves of small seedling avocado trees in a nursery at Ancon, C. Z., by Mr. Zetek, and further search was rewarded in April and May, 1919, when "wild" avocado fruits, the seeds of which contained Heilipus larvæ, were collected at the large avocado plantation at Frijoles, C. Z. These fruits came from large trees growing wild at the edge of a plantation and at a considerable distance from the cultivated, bearing trees. Attempts to determine the previous history of these "wild" trees were unavailing. Infested fruits were brought to the Board of Health Laboratory at Ancon, and the adults reared from them did not differ from the two collected in 1918 or from the large specimen which had been sent to the National Museum by Mr. F. H. Jackson about 1912. From the occurrence described above and from the date of the last-mentioned specimen it would appear that the species is endemic in Panama, but there remains a possibility that it

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[1] Resigned Nov. 3, 1919.

[2] BARBER, H. S. AVOCADO SEED WEEVILS. *In* Proc. Ent. Soc. Wash., v. 21, no. 3, p. 53–60, pl. 2. 1919.

[3] A very large specimen was received at the United States National Museum about 1912 from Las Cascadas, C. Z. (F. H. Jackson, collector), but it was not treated in the paper by H. S. Barber cited above because of absence of data definitely associating it with avocado. Other close relatives with similar habits will undoubtedly be found in other avocado-growing regions of tropical America.

[4] This interception was made by Mr. O. K. Courtney, Port Inspector of the Federal Horticultural Board at New Orleans, La., in October, 1919. *H. perseae* Barber was found in an avocado seed in the baggage of a steamship passenger arriving at New Orleans from Cristobal, C. Z.

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might have become established there long ago through the importation of avocados or their seeds from some other part of the American Tropics.

Miscellaneous information regarding the habits of the various stages of the weevil was obtained in rearing it. Some notes regarding its economic importance and distribution were also made.

At Frijoles only the "wild" fruits were infested, 17 out of 40, or 42.5 per cent, of such fruits containing from 1 to 4 larvæ. Out of over 200 cultivated fruits examined here not one was found infested. Fruits infested with Heilipus larvæ have been found on fruit stands in Panama City and Colon, in the Republic of Panama, and at Gatun and Ancon, in the Canal Zone. The only information obtainable in these cases regarding the origin of such fruits was that they came either from the Canal Zone or neighboring parts of the Republic of Panama. From the data at hand the species seems to be limited to the "Canal Zone region," though there is little doubt that it occurs over a much wider area.

EGG PUNCTURES AND LARVAL HABITS

The egg punctures are somewhat crescent shaped, about 4 mm. long, with the ends blunt. In a general way they resemble those of the plum curculio. As many as 10 were found on a single fruit, but in 8 of these the eggs had been crushed by the growing fruit and in 2 young larvæ had hatched. No eggs were found, but from the examinations of infested fruits it is evident that the eggs are laid at the junction of the skin of the fruit and the pulp. The exact time that oviposition takes place is not known, but from the evidence at hand it is when the fruit is between one-half and three-fourths mature.

After hatching, the larvæ often wander through the pulp before entering the seed, thus rendering a considerable part of the fruit inedible, especially where more than one larva occur in it. Once the larvæ enter a seed they confine their activities to it. Mr. Barber has called attention to the fact that seeds infested with *H. lauri* and *H. pittieri* do germinate if the embryo has not been injured by the tunnelling of the larvæ, and the same thing has been observed in the study of *H. perseae*; but when a seed becomes infested with two or more larvæ, it is usually so badly riddled that it can not germinate. Furthermore, seeds infested with Heilipus larvæ seem to be subject to the attacks of several kinds of "dry rots" which follow along the tunnels, invade the embryo, and kill it. Likewise, these fungi, at least under laboratory conditions, seem to be indirectly responsible for the death of a considerable number of larvæ and pupæ.

No natural migration of larvæ from one seed to another, even when these seeds are massed together, has been observed, but half-grown larvæ taken from infested seeds immediately tunnelled into uninfested ones when these were provided.

The duration of the larval stage was not determined, but indications are that it is not less than three months.

PUPATION

When the larvæ are full grown, instead of leaving the seed they hollow out a large spherical cell in which they pupate. Three such cells have been found in one-half of a large avocado seed, and four adults have been reared from a single seed. This is probably as large a number of adults as can be obtained from one seed because of the quantity of food eaten by the larvæ and because of the fact that the larvæ tunnel freely from one cotyledon of the seed to the other. The minimum duration of the pupal stage is 12 to 15 days.

HABITS AND INJURY BY ADULTS

The adults, on transforming from the pupal stage, rest in the pupal cell from two to four days and then cut their way out. At the time they come from the pupal cell the adults are decidedly reddish in color, with six prominent yellowish spots, as given in the technical description. The reddish color becomes darker with age and is finally blackish in reared individuals that live over two months.

The adults readily drank water that collected on the sides of the glass cages to which they were confined. They ate and seemed to flourish on half-ripe fruit, young leaves, and stems of avocado and on fresh avocado seeds. In one case an individual that had been starved for a week ate a few holes in guava leaves.

Injury to the fruit and to the leaves and stems is shown in Plate 7, C, and in Plate 8. An interesting thing about the fruit injury is that the outer skin was first eaten off; then, as the surface of the pulp became dry a day or so later, this in turn was eaten off, the result being that within a week holes almost ½ inch deep were eaten out. On the young stems the bark layers were gnawed off first and the woody areas were then eaten through, so that all the parts above the injury collapsed. Similar injury was done to the petioles of the leaves. In inspection work at the Plant Inspection House of the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, at Washington, D. C., avocado bud wood has repeatedly been received from Guatemala showing insect scarring similar to that caused by the light feeding of *H. perseae* on young stems. This injury on the Guatemala bud wood may have been the feeding injury of *H. pittieri* that had "healed over." In practically every way the feeding habits of *H. perseae* are similar to those of *H. lauri* as recorded by Barber.

The shortest time that any individuals of this new species (*H. perseae*) remained alive was 10 days, all of them without food. One male without food but with copious and regular supply of water remained alive 23 days. It was observed that when individuals were kept in dry cages they soon died, even in the presence of food. The longest time any individual remained alive was 116 days, this being a female.

Although five individuals (two males and three females) were kept together 35 days, no mating was observed, nor did oviposition take place on the half-ripe fruit that was provided for this purpose.

GENERATIONS

The apparently long duration of the larval stage and the known longevity of the adults indicate that there is but a single generation in a year. If this is true, then it is a long-drawn-out generation, for, from the material obtained at Frijoles, adults emerged over a period of 40 days, and in several cases a month elapsed between the emergence of the first and last adults from the same seed. It is probable, however, that breeding is controlled in the Tropics more by the activities of the host plants in supplying the proper conditions for oviposition.

CONTROL

The control of all three species of the genus Heilipus now definitely known to infest avocado seeds is comparatively simple, because pupation takes place inside the seed. It consists of gathering up and burning the fallen fruits and seeds. This control may be complicated, however, by the presence of "wild" trees that are not readily accessible or easily eliminated. In such cases it may be possible to protect cultivated fruits by arsenical sprays, for the adults feed freely on the leaves and doubtless in the field drink considerable water off the leaves when these are wet.

DESCRIPTION OF HEILIPUS PERSEAE

Heilipus perseae Barber, n. sp. (Pl. 7, A, B.)

Closely related to *H. lauri* Boh., but more robust; the squamose fascia of elytra larger, and, in addition, a similar squamose area on the sides of the pronotum. The rostrum is short in both sexes, and the mesosternum is not prominent. The legs are also much shorter than in either *H. lauri* or *H. pittieri*.

Ovate, shining, rufopiceous, clothed sparsely with scales which are white on legs and under surface, pale ochreous in the seriate elytral punctures, and darker ochreous on thoracic and elytral fasciæ, the marginal scales of which appear paler. Frontal fovea deep; eyes much larger than in *H. lauri* and separated above by less than half the width of rostrum; the latter shorter (eye to apex) than the pronotum in both sexes. Pronotum very coarsely sparsely punctate, median line impunctate but not elevated; lateral squamose areas irregularly oval, usually a little produced downward in the anterior constriction, but rarely extending to basal or apical margins. Scutellum small, subtriangular, convex, impunctate, polished. Elytra sparsely seriately foveolate, the foveæ densely squamose; two large squamose areas in same position as the small ones in *H. lauri*, the apical fascia usually extending from side margin to suture, but sometimes nearly divided at suture. Mesosternum a little produced but not projecting beyond coxæ. First and second ventral segments feebly impressed at middle in the female, a little more strongly impressed in the male. Tibial claws short and stout. Length (rostrum excluded) 11 to 15.5 mm., width 4.8 to 5.7 mm. Length of rostrum, males 2.9 to 3.4 mm., females 3.2 to 4.1 mm.

The sexes are extremely difficult to distinguish, unless the tip of the aedeagus or the "palps" of the ovipositor can be seen. Nine males and seven females are before me, all having been reared from avocado seeds at Frijoles, C. Z., by Mr. H. F. Dietz, during May, June, and July, 1919, except a male taken at Ancon, June 20, 1918 (J. Zetek No. Z1084), and a large undated female (the allotype) from Las Cascadas, C. Z., received from F. H. Jackson about 1912.

Type, allotype, and 14 paratypes, United States National Museum No. 22586. One paratype retained by Federal Horticultural Board and one paratype sent to Mr. Geo. C. Champion.

PLATE 7

Heilipus perseae:

A, B.—Adult, paratype. × 5.

C.—An avocado fruit (reduced) showing feeding injury by the beetles.



A



B



C



PLATE 8

Heilipus perseae:

Leaves showing the injury done by five beetles in 48 hours.

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PLATE 9

Heilipus perseae, mature larva:

A.—Ventral face of ventral mouth parts.
B.—Anterior part of head from above.
C.—Lingua, hypopharynx, hypopharyngeal bracon, and dorsal (buccal) face of maxilla.
D.—Dorsal face of mandible.
E.—Epipharynx.
F.—Ventral face of mandible.
G.—Head capsule from above.
H.—Thoracic spiracle from outside.
I.—Mature larva.

Drawings, from studies, by Dr. A. G. Böving.

A
B
C
D
E
F
G
H
I
Heilipus persese Barr.

STUDIES IN MUSTARD SEEDS AND SUBSTITUTES: I. CHINESE COLZA (BRASSICA CAMPESTRIS CHINOLEIFERA VIEHOEVER)

By ARNO VIEHOEVER, *Pharmacognosist in Charge*, JOSEPH F. CLEVENGER, *Assistant Plant Histologist*, and CLARE OLIN EWING, *Assistant Pharmacognosist, Pharmacognosy Laboratory, Bureau of Chemistry, United States Department of Agriculture*[1]

INTRODUCTION

Shortly after the outbreak of the recent great war many products which previously could be obtained from European countries were no longer available, and as a result importers were obliged to seek other sources of supply. One of the products thus affected was mustard seed. It was soon apparent that much of the seed offered for entry as mustard was quite different not only in quality but also in general appearance and condimental character from that which had usually been imported. Some of the shipments, for example, of Chinese mustard (*Brassica juncea* (L.) Cosson), while not so satisfactory as the mustards formerly recognized, consisted of seeds with condimental and medicinal qualities which made them useful as substitutes. Others, consisting of Japanese mustard (*41*)[2] (*Brassica cernua* Thunb.), proved to be very valuable material. It is probably grown under more favorable climatic conditions and is evidently collected more carefully than the Chinese seed.

Seeds from some other Brassica species which possessed no medicinal or satisfactory condimental value, however, were imported (*1, p. 469; 45; 46; 48*), and among these was the one to which this article has reference. The seed was first called to the attention of the authors because it had been imported in large quantities as rape seed and subsequently was introduced into interstate trade as mustard seed. Its appearance was rather bright, though not shiny, and resembled in a way yellow or white mustard (*Sinapis alba* L.) (*31, p. 379*). On account, however, of its peculiar earthy flavor and lack of the pungency characteristic of mustard, it did not meet with the unqualified approval of the trade.

[1] During the progress of the botanical work the authors obtained valuable assistance from the Bureau of Plant Industry, United States Department of Agriculture, and desire to acknowledge especially the help of Messrs. Brown and Hillman, of the Seed-Testing Laboratories; Mr. Shoemaker, of the Office of Horticultural and Pomological Investigations; Messrs. Fairchild, Bisset, Skeels, Stuntz, and Rankin, of the Office of Foreign Seed and Plant Introduction; Messrs. Coville and Blake, of the Office of Economic and Systematic Botany; and Messrs. Swingle and Tanaka, of the Office of Crop Physiology and Breeding Investigations. Prof. Trelease, of the University of Illinois, also kindly gave his advice. For valuable assistance in connection with the chemical work appreciation is due to Mr. Burnett, formerly of the Oil Fat, and Wax Laboratory; to Mr. Gowen, formerly of the Baltimore Food and Drug Inspection Station and especially to Mr. Bornmann, of the Chicago Food and Drug Inspection Station, all of the Bureau of Chemistry, United States Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 137–139.

CLASSIFICATION

IDENTIFICATION

While a preliminary study seemed sufficient to exclude the seed from the group of true mustards,[1] much difficulty was encountered in definitely identifying it. The material had evidently not been imported before, at least not in recent years, nor could similar authentic material be located in this country in any of the larger museums. Since the information on the subject in the literature was contradictory, insufficient, or entirely lacking, extended studies were undertaken to determine the macroscopic and microscopic characteristics of the seeds, as well as the chemical composition and certain physiological characteristics of the volatile oil. Plants were also grown to maturity, and the characistics at the different stages of growth were determined. These experiments were correlated with data in the literature, as a result of which identification of the seeds as those of Chinese colza, *Brassica campestris chinoleifera*, n. var., was made possible. It should be mentioned here that Chinese colza was first classified by us (*1, p. 469; 45; 46*) as *Brassica campestris chinensis oleifera*, n. f. Upon suggestion of Messrs. Blake and Coville the name was changed to *Brassica campestris chinoleifera*, n. var., in order to avoid the use of a polynomial.

TAXONOMY

Some confusion exists concerning the nomenclature of Brassicas, the description of them in many instances being inadequate. This is especially true of the oriental species, of which the seed in question is a representative. Linnaeus (*26, p. 281*) described *Brassica chinensis* (Pl. 19, A) as a plant having stem-clasping leaves and slightly compressed siliques. It is obviously of the *Brassica campestris* type (Pl. 19, B).

Iinouma (*18*) described among other vegetables two plants which he called, respectively, Aburana (oil vegetable) and Tona (Chinese vegetable). Tanaka and Ono (*18*) identified Aburana as *Brassica chinensis* var., and Tona as *Brassica chinensis* L.

Ito and Matsumura (*19, p. 299–301*) include *Brassica chinensis* L. and *Brassica orientalis* Thunb. under the species *Brassica campestris* var. *chinensis* T. Ito. Kondo (*21, 22*) evidently accepted this classification and described Aburana, used for oil production, and (*4*) other forms, used for greens, as *Brassica campestris chinensis* T. Ito. Makino (*18*), apparently unaware of Ito's classification or Kondo's earlier work, identified both Aburana and Tona as *Brassica campestris* L. var. *chinensis* Makino. According to Georgeson (*16*), Abura-na, Nutum-na, and Chirimen-na[2] are Japanese names for Chinese cabbage, *Brassica chinensis* L.,

[1] Mustard seed is the ripe seed of *Sinapis alba* L. (white mustard), *Brassica nigra* (L.) Koch (black mustard), *Brassica juncea* (L.) Cosson, or the varieties or closely related species of the types of *Brassica nigra* and *Brassica juncea*, for example, *Brassica cernua* Thunb. (*42*).

[2] Free translation according to Georgeson: Na means green; abura, oil; nutum, rape seed; and chirimen, crape, referring to the crimped leaves of certain varieties.

which he, in agreement with Miquel (*29, p. 74–75*), considers identical with *Brassica orientalis* Thunb. He states (*16, p. 652*):

> No other vegetable of this class is so universally grown, or is represented by so many varieties. It is a kind of rape which has been transformed by cultivation. Certain varieties of it are grown only for their seed, from which an oil is expressed, formerly much used as lamp oil.

Judging by the illustrations and the very brief description given by these authors, and considering the great variations observed in plants grown from Chinese colza seed, it appears quite probable that both Aburana and Tona may be included in the series of plants treated as *Brassica campestris* var. *chinensis* by Lund and Kiaerskou.

Lund and Kiaerskou (*28, p. 166–167*), who carried on extensive growing and crossing experiments, classify under the name *Brassica campestris* var. *sativa annua chinensis* two forms of Chinese vegetables, Pe-tsai and Pak-choi.

Prain (*32, p. 42, 45*) gave to Pak-choi (Chinese cabbage), which he found growing on the Indian plains, the name *Brassica chinensis* L., including in this species also the plants described under the following names: *B. chinensis* L. var.; *B. campestris* Forbes and Hensl. in part, not of L.; *B. juncea* Forbes and Hensl. in part, not of H. f. et. Th.; *B. oleracea* L. var. *chinensis* Prain; *Sinapis brassicata* L.; *Pak-choi* Vilmorin; *Pak-tsoi* Roxb.; *Yea-tsoi* Roxb.

Vilmorin (*49, p. 491*) classifies under *Brassica chinensis* L., in addition to Pakchoi and Pe-tsai, a third form of less cultural interest which has almost entire leaves with narrow petioles.

Stuart (*38, p. 73*) classifies only Pe-tsai or Pai-tsai under *Brassica chinensis* and states that it is a most common variety of *Brassica oleracea*. He points out, however, that Yu-tsai,[1] undoubtedly *Brassica rapa*, yielding rape seed from which rape-seed oil is manufactured, is also called *Brassica chinensis*, possibly on account of its economic prominence in China.

Bailey (*3*) refers to Pak-choi and Pe-tsai as two different species, calling the first *Brassica chinensis* L., and the latter *Brassica pe-tsai*. He considers that Linnaeus' description for *Brassica chinensis* answers best for Pak-choi.

Gagnepain (*15*) has renamed Pe-tsai, classified by Loureiro (*27, p. 400*) as *Sinapis pekinensis*, *Brassica pekinensis* (Lour.) Gagnepain. Skeels (*43, p. 21*), evidently unaware of Gagnepain's classification, renamed the same form *Brassica pekinensis* (Lour.) Skeels.

Duthie and Fuller (*10, p. 33–34*) give the name *Brassica chinensis* to a plant with many characteristics of *Brassica juncea*, but they point out that they consider *Brassica chinensis* Duthie and Fuller synonymous with *Sinapis chinensis* L. The choice of the name *Brassica chinensis* is unfortunate for a plant with characteristics of *Sinapis chinensis* L. and apparently identical with or closely related to *Brassica juncea* (L.) Cosson.

[1] Dr. Yamei Kin, familiar with China and its agricultural products, suggested that the material which the authors considered as Chinese colza was Yu-tsai. However, since the seeds examined by the authors differed from samples obtained as Yu-tsai from China, it appears that this name is not definite.

TERMINOLOGY

SCIENTIFIC NAME

While it is believed that the plants grown from material in the Pharmacognosy Laboratory (Pl. 18, A), show characteristics typical of the plant described by Linnaeus as *Brassica chinensis*, and while they apparently agree rather well, so far as the general morphology of the plant is concerned, with Pak-choi (Pl. 15, C), there are certain differences, especially in the seeds, from Pak-choi as well as Pe-tsai. The seeds of Pak-choi and Pe-tsai were generally found to be smaller, more spherical, and usually of a brown color. As a rule, they show even less marked reticulations than the brown seeds of the authors' material. The most striking differences observed in the plants is the lack of broad petioles (see also Vilmorin's description of one form) and the failure to form heads in the rosette stage, so strongly developed in Pak-choi and especially in Pe-tsai (Pl. 15, A). These differences, however, while distinct, are not so marked that they might not be considered to fall within the latitude of species character. It would, therefore, seem that the laboratory material might be classified as a variety of the type species *Brassica chinensis* L. were it not for the following reasons. The description which Linnaeus gives is very brief, in fact so brief that much of the confusion in the use of this specific name by different authors is probably due to this limited species description. Bailey points this out, giving still another instance where the name *Brassica chinensis* has been used, evidently not correctly (*3, p. 543*):

> It is impossible to determine whether this particular plant [Pak-choi cabbage] is the one that Linnaeus meant to distinguish by his *Brassica chinensis*, but it best answers the description in his Amoenitates (Vol. IV). In Linnaeus's herbarium is a Brassica marked "chinensis" in his own handwriting, but it shows purple fls. and has lyrate-lobed lvs., whereas Linnaeus described his plant as having yellow fls. and cynoglossum-like lvs., probably not the original.

Linnaeus's description, nevertheless, indicates the close relation to *Brassica campestris*, and Lund and Kiaerskou showed this close relation by classifying both Pak-choi and Pe-tsai as *Brassica campestris* var. *annua sativa chinensis*.

Bailey (*2, p. 188*) takes a different stand:

> In common with all members of the genus Brassica, or cabbage and mustard tribe, these Chinese plants are much confused respecting their botanical characters. Recent writers have referred all the Chinese cabbages to *Brassica campestris*, the rutabaga; but one who studies the plants carefully, both from herbarium and living specimens, can not long hold this opinion. The genus Brassica divides itself naturally into two groups—the cabbages and rape, characterized by thick leaves, very glaucous-blue herbage and long flowers which are creamy white, and the mustards, with thinner and green or lightly glaucous herbage and small, bright yellow flowers. The Chinese cabbages belong to this latter group rather than to the former. Their flowers are those of the mustards, and I have no hesitation in removing the plants from *Brassica campestris*.

He thus takes a different stand from all other botanists who have given attention to these "Chinese cabbages and mustards." The authors of this paper also disagree with Bailey's viewpoint and classification on the basis of a rather extended investigation reported in the following paragraphs. There is no doubt in their minds that the so-called Chinese cabbages are not mustards but belong to the colza group, *Brassica campestris* L.

Concerning *Brassica campestris* L., Prain (*32*) states:

> From the standpoint of commerce it is a matter of supreme indifference whether campestris, napus, and rapa be treated as separate species or subspecies of one and the same species.

Consequently, in his systematic synopsis he proposes a number of groups:

> (1) *Brassica oleracea*, cabbage group; and (2) *Brassica campestris* Linn. ampl. Subspecies A, *campestris* (sp. Linn.), representing the colza group, subspecies B, *napus* (sp. L.) representing the rape group, and subspecies C, *rapa* (sp. L.) representing the turnip group.

As to the close relationship of the respective forms, Bailey states (*3*, *p. 544*) that he—

> ound no difficulty in crossing cabbage-kale-cauliflower and others.

Lund and Kiaerskou, especially, showed by extensive crossing experiments the close relationship of *Brassica oleracea*, *Brassica campestris*, and *Brassica napus*. Notwithstanding this close relationship, however, it appears necessary to go further than Lund and Kiaerskou (28) in the classification of some of these forms, for instance in the classification of Pak-choi and Pe-tsai. Bailey states (*2*, *p. 189*)—

> there is even good reason for separating the two types of Chinese cabbage . . . into two species, for they differ widely in their leaf characters and pods; and the former [*Brassica pe-tsai*] is truly annual, while the latter [*Brassica chinensis*] is evidently normally biennial.

Although the authors did not study these forms extensively, Shoemaker has shown that they can be readily crossed (Pl. 15, B) and therefore should not be considered as having species character (*4*). It is at present impossible to state definitely the relationship of Chinese colza to these forms. Since it has greater similarity to Pak-choi than to Pe-tsai, it appears not unlikely that Chinese colza and Pak-choi have developed from one common stock. Pe-tsai may present a further modification of Pak-choi, since, it is said, plants with narrower petioles may develop from Pe-tsai seeds. Pending further collection of data on Pe-tsai and Pak-choi the following classification, based on that of Bailey, Lund and Kiaerskou, Gagnepain, and others, appears satisfactory for the separation and identification of these horicultural and oil-yielding forms:

1. Pak-choi, *Brassica campestris chinensis* T. Ito.
2. Pe-tsai, *Brassica campestris pekinensis* (Lour.) Viehoever.
3. Chinese colza, *Brassica campestris chinoleifera* Viehoever.

This classification appears the more satisfactory, at least so far as Chinese colza is concerned, since it indicates clearly the very close relationship to Indian colza, *Brassica campestris* var. *glauca* Watt. This relationship is evident from botanical characteristics of the plant, and especially from the morphological and anatomical characters, as well as from chemical characters of the seeds of both Chinese and Indian colza.

POPULAR NAME

The popular name "Chinese colza" has been selected on the basis of the findings enumerated. Furthermore, it appears preferable to "China" or "Chinese cabbage," names often used for similar seeds, especially for Pe-tsai or other related horticultural varieties. Tracy (*39, p. 603*) states:

> The Chinese cabbage of this country is a wholly different species from the common cabbages. Chinese cabbage does not form a compact and rounded head. . . .

Georgeson (*16, p. 652*) states:

> The term cabbage is a misnomer, as its resemblance to that vegetable is quite remote. The plants are merely bunches of large, smooth, more or less spreading leaves, with broad fleshy midribs. They do not bear their leaves on a well defined stem, as do the cabbage, the kale, etc., but look more like the Cos lettuce, the leaves having their origin at the surface of the ground.

Learning also that certain forms of *Brassica oleracea*, apparently peculiar to China, are grown there, the authors felt that the name "Chinese cabbage" could properly be applied only to those.

The authors' form, although rather closely related to *Brassica oleracea*, is primarily an oil-yielding form which does not head and which deserves the designation "cabbage" even less than Pe-tsai and Pak-choi, both more or less heading forms. Some consideration was given the name "Chinese yellow rape," as the seeds resemble rape seeds in a way and yield a fixed oil similar to rape oils. In order to avoid confusion in horticultural nomenclature and to protect the agriculturist, however, it was considered advisable to adopt the more specific name of "Chinese colza."

BOTANICAL STUDIES

DESCRIPTION OF SEEDS

The seeds (Pl. 10, A, B) of Chinese colza, *Brassica campestris chinoleifera* Viehoever, are yellow or brown, and, if immature, green in color. In mass they have a dull yellow color, due to the preponderance of yellow seeds. In form they are somewhat compressed, oval, and usually with distinct ridges on one side. The size varies from 1.4 to 2.6 mm. in the long axis. The weight varies from 1.4 to 6.4 mgm., with an average weight (based on 1,000 seeds) of 2.865 mgm. The weight of 500 mils (quantity filling a 500-mil measure cylinder to the 500-mil mark) was 352 gm.

The surface usually appears smooth (Pl. 10, A, B) but under a hand lens shows very weak reticulations on the yellow seed and more distinct, but by no means prominent, reticulations on the brown seed (Pl. 10, C, D).

In cross section under the microscope the epidermis of the seed coat (Pl. 10, E, F, *a*) is striated tangentially, does not show any cell structure as in the mustard seed, and is about 5 microns thick. It does not swell appreciably when moistened and does not show crosses with polarized light. The sclerenchymatic palisade cells (Pl. 10, E, F, *c*) vary more in height in the brown seed than in the yellow. This explains the presence of the more pronounced reticulations in the brown seeds. For the yellow seed the height is almost uniformly 20 microns, while the average for the brown seed is about 25 microns, with a maximum height of 31 microns. The limits found for all the seeds were 15 to 31 microns high by 8 to 15 microns wide. The cell walls are strongly thickened at the base and sides, and the inner walls are smooth. The lumen contains no color substance. The parenchyma, always developed to one or more rows in the *Brassicas* (*40, p. 615*), is compressed to such an extent that it appears to be almost entirely lacking (Pl. 10, E, F, *b*). In *Brassica nigra* one row and in *Sinapis alba* two rows of parenchymal cells are clearly visible, even in the mature seeds.

The parenchyma (Pl. 10, E, F, *d*), located below the palisade cells, consists mainly of one row of cells which in the yellow seeds contain no color substance but in the brown seeds are filled with pigment. The endosperm (*e*) is characterized by the protein layer, a row of cells usually one cell wide, but occasionally two cells wide, the cells varying in height from 15 to 21 microns and in width from 15 to 42 microns and containing protein masses. The tissue (*f*) located below this layer is composed of several layers of parenchyma cells which, especially in the mature seeds, are strongly compressed. The embryo consists of two cotyledons folded in a characteristic way around the radical. The tissue is parenchymatic or meristematic. The cells which form the cotyledon tissues are not characteristic except that they contain globules of fatty oil, protein masses, and, especially in the immature state, a limited number of small starch grains which range in size up to about 6 microns in diameter. Experiments to locate the glucoside as a crystalline body have been unsuccessful. Studies to locate the enzym and glucoside microchemically in the cells are being undertaken.

DESCRIPTION OF THE PLANT

Experiments in the growth of selected yellow and brown seeds were made under greenhouse and field conditions. The field experiments were made at Arlington, Va., during the summer of 1916, and at Yarrow, Md., during the summer of 1917. The laboratory records, so far as differences in stages of growth are concerned, are more complete for the plants grown in the greenhouse.

The plants in all stages of their growth were generally smooth, with entire leaves. The young leaves, however, especially if grown in humid atmosphere, were more or less hairy, mainly on the margins (Pl. 11). In older leaves hairs were observed only occasionally. It was noted that isolated plants showed variations in the lobing, the leaves in some instances being deeply notched (Pl. 13). Experiments are being carried on to determine the latitude and significance of these variations. The appearance of some of these lobed leaves was very similar to that described for *Brassica napiformis* Bailey (*Sinapis juncea* var. *napiformis* Paill. and Bois), an observation which has much significance in view of Bailey's statement (*3*) that—

> it is nearly related to pak-choi, and it may have sprung from the same species; but it is clearly distinguished by its sharply toothed lvs. . . .

In the early stages the cotyledons had the same general appearance but were somewhat larger and thicker than those of the following mustards, *Brassica nigra* (L.) Koch, *Brassica juncea* (L.) Cosson, and *Brassica cernua* Thunb.[1] They were about 1 cm. long and 1 cm. broad, exclusive of the petiole, and are heart-shaped and smooth (Pl. 11). The first leaves were obovate, variously toothed, and somewhat crenate, and were hairy, especially on the margin if the seedling had been grown in very humid atmosphere. The leaves had a long petiole and a mid vein extending at least one-third of the length of the blade (Pl. 11).

In the late rosette stage (Pl. 14, 15) the leaves were arranged in a loose cluster, the wings of the leaf extending along the greater portion of the petiole, with the margin of the leaf more or less wavy and almost entire. The time required for the development of the full-grown rosette stage varies with the conditions for growth, being on the average about two months when grown under normal conditions in the field and about three months in the greenhouse. This period is materially shortened when there are conditions decidedly unfavorable for growth, such as insufficient nutriment, insufficient moisture, or too high temperature.

The early flowering stage (Pl. 16, 17) is characterized by a few erect branches up to 1 foot in length. The early stem leaves are similar to the rosette leaves, being almost entire, and obovate with long petioles. The upper stem leaves are variously stem-clasping, entire, somewhat glaucous and somewhat lanceolate acuminate. Many of the leaves of the secondary stems are not stem-clasping. The mature plant reaches a height of about 2 or 2½ feet, branching, and often showing an enlarged stem base (Pl. 16, A).

The flowers (Pl. 17, B), which are somewhat larger than those of *Brassica nigra*, *B. juncea*, and *B. cernua*, are in dense wide corymbs, 1½ inches

[1] Plants of *Sinapis alba* need not be considered in the comparison, since they are distinctly different from the other forms and can readily be recognized by such characters as the abundance of typical hairs on the entire young plant, as well as on the later plants, especially the pods, which themselves are readily distinguished by their typical shape.

long and 2 inches across when the flowers are open, subsequently elongating into racemes 6 to 18 inches long, with pedicels ½ to 2½ inches long in the extreme, slender, and without bracts or bractlets. The long pedicel particularly distinguishes the flower from the flowers of the mustards, which rarely have pedicels longer than ½ inch (17). Otherwise the flowers do not differ essentially from the general type of the genus Brassica.

The mature fruit pods (Pl. 18, B) are 2-valved, and are 2 to 3 inches long, including the beak. The beak of the pod is rather thickly conical and from 0.4 to 0.8 inch long. The valves are convex, rigidly leathery, rather finely nerved, and beaded opposite the seeds. A cross section of the pod is broadly elliptical throughout the entire length and about ¼ inch thick across the long axis. In some of the pods both yellow and brown seeds have been observed, giving evidence that the yellow and brown seeds are only variations in the same kind of seed. An examination of plants grown from brown and yellow seed will also prove this statement to be correct (Pl. 12, A). The green seeds are immature, as is indicated by the abundance of small spherical starch grains occurring in the cotyledons. From 8 to 12 seeds are found under each valve of a fully developed fruit pod.

BOTANICAL CONCLUSIONS

On the basis of the descriptive data given, the authors' material must be classified with the colzas and rapes rather than with the true mustards. While some of the characteristics observed would have only a limited diagnostic value if taken alone, they serve as additional means for the differentiation. Considered together, they make the proper classification the more certain. The botanical characteristics may be briefly recapitulated as follows.

SEEDS

1. As is typical of the colza group, the seeds are rather smooth. True mustards, except *Sinapis alba* L., show generally a more pronounced reticulation of the seed coat.

2. As in the case of Indian colza (*Brassica campestris* var. *glauca*), the seeds are more or less flat. True mustards are generally spherical, except *Brassica besseriana* Andrews, which has large brown seeds of more or less oval shape. Many rapes and Brassicas other than mustard, however, are also spherical.

3. A very pronounced ridge can be found in almost every seed of the Chinese and Indian colza, while it is scarcely developed in the mustard seeds, with the possible exception of *Sinapis alba*.

4. The swelling and polarizing epidermis is lacking in the Chinese colza seed, as usually also in other seeds of the colza group. While not so distinct or appreciable in certain forms or variaties of *Brassica juncea*, the swelling of the mucilaginous epidermis and the polarization are

especially pronounced in *Brassica nigra*, *Brassica besseriana*, and *Sinapis alba*. Swelling, however, has been observed in cabbage seed, *Brassica oleracea bullata gemmifera* (*40, p. 615 and table*).

5. The form and size of the palisade cells of the seed coat are similar to those of the general type found in the colza group and differ more or less strikingly from the true mustards.[1]

PLANTS

1. The tendency to rosette-like growth of plants in the early foliage stage, great in plants belonging to the colza group, was also observed in the authors' material. With the exception of certain variations of *Brassica juncea*, the authors have not observed a similar tendency in mustard plants.

2. The almost entire lack of hairs, especially pronounced in more advanced plants, has been noted on the plants studied, as well as on other plants of the colza group, a possible exception being *Brassica rapa*, reported by Bailey. In contrast, the plants of mustards are more or less distinctly hairy.

3. The upper leaves of the flower stalk are stem-clasping, as is general in the colza group; no distinctly stem-clasping leaves have been observed in plants of true mustards.

4. The pedicels (stalks of the flowers) of Chinese, as well as those of other colzas, average well over ½ inch in length, while those of the mustard flowers average less than ½ inch.

5. The greater length of the pods of Chinese and other colzas, often more than 2 inches, including the beak, frequently distinguishes them from the mustards, which, as a rule, have shorter pods, averaging usually less than 2 inches. Bailey (*2*), however, reports short pods for Pe-tsai.[2]

CHEMICAL STUDIES

GENERAL COMPOSITION OF SEEDS

The chemical studies included the general composition of a number of samples of the seed, as well as a more detailed examination of the fixed and volatile oils. Table I shows the composition of typical samples of the seed.

Judging from the composition of the seed and the low amount and character of the volatile oil yielded, the authors believe that the pressed oil cake will be a very good feeding material.

[1] For further details and comparison with other cruciferous seeds, the key given in Winton (*51, p. 173–189*) may be consulted.

[2] For further information and comparison, see Bailey (*3*), Howard et al. (*17*), and textbooks on taxonomy.

TABLE I.—*Analyses of seeds of Chinese colza (Brassica campestris chinoleifera Viehoever)*[1]

Sample No.	Moisture.	Ash.	Ether extract.[2]	Protein (N×6.25).	Reducing substances as starch by acid hydrolysis.	Crude fiber.	Volatile oil (crotonyl isothiocyanate).[3]	Iodin No. on ether extract (Hanus).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	4.12	8.64	39.89	22.76	11.41	3.94	0.43	.....
	4.11	8.62	40.22	22.76	11.34	3.86	.43	.....
2	4.07	5.51	42.19	24.08	11.26	3.83	.57	99.8
	4.07	5.45	42.10	24.78	11.29	3.93	.54	99.8
	.....	.....	.....	.....	.....	.....	.....	99.4
3	4.07	8.45	40.25	22.72	11.64	3.99	.49	99.6
	4.04	8.49	40.30	22.85	11.71	4.33	.50	.....
4	3.86	8.41	40.35	22.89	11.70	3.96	.47	98.6
	3.88	8.39	40.42	22.76	11.65	4.03	.48	.....
5	5.25	5.14	42.4	24.38	.....	4.09	.52	.....

[1] Analyses of samples 1 to 4 were made by J. H. Bornmann, of the Chicago Food and Drug Inspection Station, Bureau of Chemistry, United States Department of Agriculture. Analysis of sample 5 was made by P. L. Gowen, formerly of the Baltimore Food and Drug Inspection Station, Bureau of Chemistry.

[2] Determinations of ether extract on two other samples, made by L. B. Burnett, formerly of the Oil, Fat, and Wax Laboratory, Bureau of Chemistry, showed 48.65 and 51.40 per cent, respectively.

[3] Analyses of samples 1 to 4 were made by the method of Vuillemin (*50*); analysis of sample 5 was made by the method outlined in this paper (p. 128); other determinations were made by the method given in the Official Methods of the Association of Official Agricultural Chemists. WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted in 1912.

ISOLATION AND IDENTIFICATION OF VOLATILE OIL

A chemical investigation of the volatile oil was made in order to determine whether it should be properly classified with the volatile oil obtained from rape seed or with that of true mustard. The following procedure was employed to isolate the volatile oil:

Two kgm. of the seed in the form of a No. 20 powder were placed in an 8-liter flask; 4 kgm. of water were added; and the mixture was allowed to macerate for two hours at about 37° C. The mixture was then distilled with steam, and the distillate was saturated with salt and extracted with ether. The ethereal solution was dried over anhydrous sodium sulphate, and the greater part of the ether was distilled off, the last portions being allowed to evaporate spontaneously.

The volatile oil thus obtained had a specific gravity of 0.960 at 25°/25° C., and the distilled oil had a boiling point of between 165° and 172° C. (uncorrected) at 754 mm. These findings agree fairly well with those for crotonyl isothiocyanate, the volatile oil previously reported in rape by Sjollema (*35*, *36*). The thiourea and phenylthiourea derivatives were prepared, and their melting points and nitrogen content were determined. The results are shown in Table II.

TABLE II.—*Physical constants of allyl and crotonyl isothiocyanate*

Substance.	Specific gravity.	Boiling point.	Thiourea.		Phenylthiourea.	
			Melting point.	Nitrogen.	Melting point.	Nitrogen.
		° *C.*	° *C.*	*Per cent.*	° *C.*	*Per cent.*
Allyl isothiocyanate	1.013 to[1] 1.020 (25°/25° C.)	[1] 148 to 154	74	[2] 24.12	[3] 98.5	[3] 14.74
Crotonyl isothiocyanate	[2] .9933 (11°/4° C.)	[2] 174 (approximate).	[2] 64	[2] 21.25	[3] 53	[3] 13.60
Oil in question	.960 (25°/25° C.)	165 to 170	64	20.74	54 to 55	13.20

[1] U. S. P. IX (1916). [2] Sjollema (1901). [3] Stein (1907).

From these data it may be seen that the oil consists largely of crotonyl isothiocyanate, which, since it is the chief constituent of volatile oil of rape, corroborates the botanical findings that the seed is related to the rapes and not to the mustards. It was noted that the crotonyl isothiocyanate did not have the odor of volatile mustard oil (allyl isothiocyanate) but had an odor suggestive of turnip or cabbage. Furthermore, it did not have the typical irritating effect of mustard oil on the mucous membrane of the nose and on the eyes nor a blistering effect on the skin.

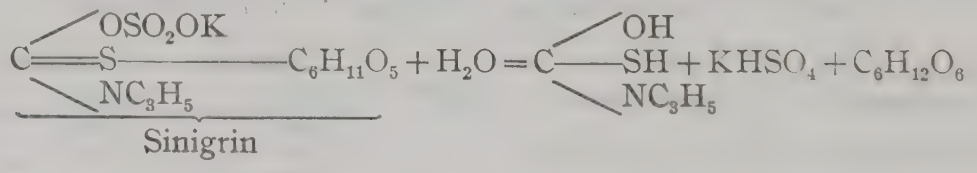
DETERMINATION OF VOLATILE OIL IN MUSTARD SEED AND MUSTARD SUBSTITUTES

In the course of this work it became necessary to determine the amount of volatile oil yielded by different varieties of mustards and mustard substitutes. Reference to the literature showed that there had been marked variation in the methods followed by different analysts in the determination of volatile mustard oils, especially in regard to the time of maceration and the conditions for distillation. Wehrmann, Wegener, Braunwarth, and Meyer (*51*) made an extended study of a number of these methods in order to arrive at a quick, convenient method for the determination of the volatile oil. In general, the studies here reported have corroborated their findings, except with respect to the effect of alcohol added before maceration (*51, p. 325*). Carles (*7, 8*) has also contributed valuable data to the solution of this problem. As a result of these studies, the following method, based largely upon that of Gadamer (*13, 14*), is recommended.

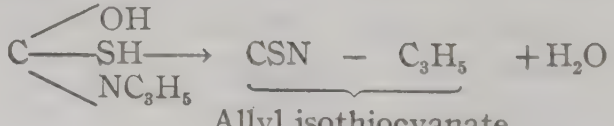
METHOD

Place 5 gm. of the ground seed (No. 20 powder) in a 200-mil flask, add 100 mils of water, stopper tightly, and macerate for 2 hours at about 37° C. Then add 20 mils of U. S. P. alcohol (95 per cent), and distill about 70 mils into a 100-mil volumetric flask containing 10 mils of 10 per cent ammonium-hydroxid solution and 20 mils of *N/10* silver nitrate solution. Mix thoroughly, stopper, and set the distillate aside overnight, heat to boiling on a water bath (in order to agglomerate the precipitate), cool, make up to 100 mils with water, and filter, rejecting the first portions. Acidify 50 mils of the filtrate with about 5 mils of concentrated nitric acid and titrate with *N/10* ammonium thiocyanate, using 2 mils of 10 per cent ferric-ammonium-sulphate solution for an indicator. Each mil of *N/10* silver nitrate consumed is equivalent to 0.004956 gm. of allyl isothiocyanate or 0.005657 gm. of crotonyl isothiocyanate.

The method is based on the hydrolysis of the glucoside by an enzym, both present in the seed. A volatile oil, glucose, and potassium hydrogen sulphate are formed.

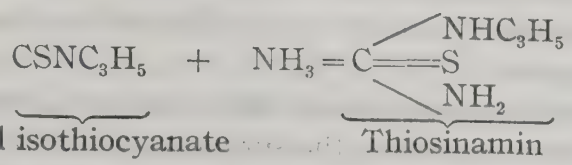


Sinigrin

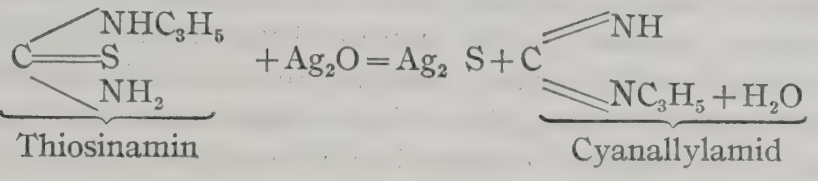


Allyl isothiocyanate

The volatile oil, readily volatile with the alcohol and water vapor, reacts with ammonia and silver nitrate. In the case of allyl isothiocyanate (the true volatile mustard oil) mainly allyl thiourea (thiosinamin) is first formed.



This reacts slowly in the cold but is completely decomposed by heating with silver nitrate, silver sulphid and cyanallylamid being formed.



Both are insoluble compounds. They are filtered off, and the silver not used up in the reaction is determined volumetrically after Volhard.

1 atom of silver = ½ molecule of the volatile mustard oil.

Other compounds may also be formed in small amounts during the process (*13*, *25*).

NOTES ON METHOD

Carles (7) suggests a smaller sample, 3 or 4 gm., in the case of partially defatted samples or others yielding especially large amounts of volatile oil.

The seed used for analysis should, if possible, be freshly ground, as the powdered material loses its strength through hydrolysis, especially if not kept thoroughly dry—at or below 7 per cent moisture, according to Carles, not exceeding 2 per cent according to Boutron (*5*).

Joergensen (*20*, *p. 9*) and van Kampen (*44*, *p. 63*) in testing rape-seed cakes recommended the addition of thymol; Brioux (*6*, *p. 262–263*) recommended the addition of sodium fluorid to the rape-seed cake when this is macerated and tested for the amount of volatile oil available. They found that bacterial action would thus be largely inhibited in the maceration and higher yields would be obtained. Brioux used 2 gm. of sodium fluorid for 25 gm. of cake and 500 mils of water; van Kampen used 10 mils of 1 per cent alcoholic thymol solution added to either 25 gm. of cake or 5 gm. of mustard seed and 300 mils of water. Joergensen used a 1 per cent alcoholic solution of thymol and also in other experiments mercuric chlorid, which, however, proved unsatisfactory. Raquet (*33*) macerated the material in an aqueous alcoholic solution, adding 15 cc. of alcohol to the mixture before and 5 cc. after maceration, thus obtaining seemingly higher yields. We (*47*) could verify his findings but are still undecided whether this higher result is due to the formation of other volatile reducing substances or, as Raquet claims, to the fact

that in the presence of alcohol during maceration no bacterial fermentation causing a loss of volatile oil takes place.

A glycerin bath may be used to secure greater uniformity in heating.

The use of ground glass joints in the distilling apparatus has been recommended in literature in order to avoid possible errors caused by the use of rubber stoppers.

To insure complete absorption of the volatile oil, the tip of the condenser should always dip below the surface of the liquid in the receiving flask. It is advantageous to have the condenser terminate in a tube of small bore. A second receiver, containing ammonia and silver solution, may be used in case the completion of the distillation is in doubt.

According to Kuntze (*25*), the mixture obtained after distillation may also be heated directly without standing to 100° C. for an hour, using a reflux condenser or a long glass tube as a condenser. Possible further formation of an urethane compound (allyl urethane in the case of allyl mustard oil) can thus be avoided.

YIELD OF VOLATILE OIL

Examination by the method just given of a number of samples of seeds obtained from Chinese colza showed that the content of crotonyl isothiocyanate varies from about 0.4 to 0.6 per cent. Various attempts were made to increase the yield of volatile oil by addition of different chemicals before maceration. Table III shows the results of these experiments.

TABLE III.—*Effects of chemicals added before maceration upon the yield of volatile oil*

Substance added to 5 gm. of sample.	Volatile oil (crotonyl isothiocyanate).
	Per cent.
Potassium hydroxid, 0.2 gm	0.08
	.11
Potassium fluorid, 0.2 gm	.41
	.54
Tartaric acid, 0.2 gm	.39
	.42
Tartaric acid, 5 gm	.05
	.05
Tartaric acid, reflux 2 hours, 5 gm	.05
	.05
Sinapis alba, as a source of myrosin, 5 gm	.52
Alcohol, 20 cc	.79
	.79
No chemical	.55
	.58

The results shown in Table III may be summarized as follows: Five gm. of tartaric acid probably destroyed the enzym and no appreciable yield of volatile oil was obtained; 0.2 gm. of tartaric acid slightly retarded

the reaction. Water alone gave results averaging about 0.6 per cent of crotonyl isothiocyanate. The addition of 20 cc. of alcohol before the maceration gave a higher percentage of volatile oil, the results reaching almost 0.8 per cent. The formation of some allyl thiocyanate (*34, p. 832*), allyl cyanid (*13*), and carbon bisulphid during the fermentation process of sinigrin has been observed in experiments where no alcohol was present. Other products may be formed in its presence and must be expected, especially in the authors' material, where no sinigrin but another glucoside is present. It has been pointed out by Kuntze (*25*) that side reactions can occur between allyl isothiocyanate and alcohol with the formation of allyl thiourethane, and it may be presumed that a similar reaction might take place between crotonyl isothiocyanate and alcohol, which will lead to erroneous but probably lower results. At the present time the data obtained are insufficient to ascribe the discrepancy to any of these causes, nor is it yet known whether, in the presence of alcohol, potassium hydrogen sulphate has also an injurious effect upon the formation of mustard oil through rendering myrosin largely ineffective by causing its coagulation (*12*). It is believed, however, that the maximum yield was obtained, since, even in the presence of large amounts of enzym (white mustard added), higher yields were not secured.

Shaking the maceration mixture at room temperature at intervals of five minutes did not hasten the reaction sufficiently to give the total amount of volatile oil in two hours.

It has been pointed out by Förster (*11*) that in the preparation of rapeseed cake the material is heated to about 70° C., and that when it is so treated a high yield of oil is obtained. In these experiments the authors were unable to verify Förster's results. In order to see whether there was any difference in the yield of oil from the brown and yellow seeds, a separation of the two was made, and determinations were made on the separated samples with the results (calculated as crotonyl isothiocyanate) shown in Table IV.

TABLE IV.—*Relative yield of volatile oil by brown and yellow seeds of Chinese colza.*

Treatment.	Yield of volatile oil.	
	Brown seed.	Yellow seed.
	Per cent.	*Per cent.*
2 hours' maceration at 37° C	0.58	0.55
½ hour's heating at 70° C., followed by 2 hours' maceration at 37° C	.44	.46

It will be observed that in both the brown and yellow seed a lower yield was obtained by the preliminary heating at 70° C. for ½ hour.

CHARACTER OF FIXED OIL, AND CERTAIN OF ITS CONSTITUENTS

It has been reported that the seed of Chinese colza is used in China for its fixed oil, and such use has also recently been made of the seed imported into this country. A study of the fixed oil showed that it was present in very large amounts, up to 50 per cent or more, and that its composition was similar to that of rape oils. It was a light yellow oil and apparently was of an excellent quality. The oil expressed from the seeds showed the characteristics given in Table V.

TABLE V.—*Characteristics of fixed Chinese colza oil* [1]

Density, 25° C	0.9097
Iodin No. (Hanus)	100.3
Saponification No.	173.8
Percentage of insoluble acids and unsaponifiable matter	96.1
Percentage of soluble acids	.07
Neutralization value of insoluble acids	172.6
Mean molecular weight of insoluble acids	325.0
Refractive index, 25° C	1.4695
Iodin No. insoluble acids	104.0
Percentage of solid acids	19.52
Percentage of liquid acids	75.82
Iodin No. solid acids	55.21

[1] Analysis by L. B. Burnett, formerly of the Oil, Fat, and Wax Laboratory, Bureau of Chemistry United States Department of Agriculture.

CHEMICAL CONCLUSIONS

1. The volatile oil yielded by Chinese colza has been identified as crotonyl isothiocyanate, an oil formerly found in rape. Crotonyl isothiocyanate is slightly lighter and allyl isothiocyanate slightly heavier than water. The boiling points of the oils and other physical constants referred to in Table II permit ready differentiation.

2. The yield of volatile oil varied from about 0.4 to 0.6 per cent, while true mustards, with the exception of *Sinapis alba*, yielded from about 0.7 per cent up to more than 1 per cent of volatile oil of different composition.

3. The fixed or fatty oil expressed from the seed showed the general characteristics of rape oils, these being slightly different from the fixed mustard oils. The iodin value, for instance, was about 100 or below in case of rape oils and the Chinese colza oil, while it was above 100 in the case of oil expressed from different mustard species. These oils were obtained under similar conditions and were unrefined. Methods of refining may change the iodin value.

4. The yield of fixed oil varied from about 40 to 50 per cent, whereas the true mustards examined usually contained less and rarely, if ever, more than 40 per cent of a fixed oil.

PHYSIOLOGICAL DATA

GENERAL PHYSIOLOGICAL CHARACTERISTICS

Chinese colza seed when chewed has an earthy and slightly pungent taste, the flavor being suggestive of cabbage or turnip rather than of mustard. When a few grams of the freshly triturated seed macerated with water have stood in a closed vessel at room temperature for a few minutes, the odor of the volatile oil formed may readily be noted. This odor gradually becomes weaker, however, and after the mixture has stood for 24 hours, more or less, the odor is largely gone and is often replaced by an odor of hydrogen sulphid. The mustards (except white mustard) give the characteristic mustard oil flavor and irritating sensation to the membranes of nose and eyes much more strongly and for a longer period. In fact, while the vapor of true mustard oil, even in very small amounts, causes great discomfort to eyes, nose, and lungs, the effect of the vapor of crotonyl isothiocyanate was by no means to be compared in intensity and hardly in character.

The ground seed, with small amounts of water added, was applied in the form of a plaster to the skin of the arm. No more than a reddening was caused after 2 or more hours of application; no blistering whatsoever was noted, and the reddening soon disappeared. It was necessary to remove plasters prepared similarly with true mustards after shorter application, and blisters were left. When applied to the skin the isolated volatile oil itself caused only a burning sensation and a temporary reddening.

PHARMACOLOGICAL ACTION

While no pharmacological experiments were made in this investigation, those of Sjollema and others may be briefly mentioned. Sjollema (*35, p. 315*) gave 0.212 gm. of the oil (crotonyl isothiocyanate isolated from rape) to a rabbit in the form of an emulsion but observed no abnormal symptoms. After about three hours the animal began to eat again, appeared entirely normal, and lived. Allyl mustard oil, isolated from black mustard, given in the same amounts and under comparable conditions, caused death to a rabbit within a few hours. The experiments were repeated with the same result. Stein (*37*) confirms Sjollema's findings in general upon the basis of a larger series of experiments, also with rabbits. He concludes that while the symptoms of poisoning are in a way the same as those caused by allyl isothiocyanate, the general toxic (resorptive) as well as the locally cauterizing properties are far less pronounced in the case of crotonyl isothiocyanate. The toxic dosis may be estimated to be 0.5 gm. of crotonyl, against less than 0.1 gm. of allyl isothiocyanate per kilo body weight.

He experimented also with a goat (24 kgm. in weight) adding to the feed of ground potato and sugar beets the following amounts of crotonyl

isothiocyanate: 1 cc. the first day, 1.5 cc. the second day, and 2 cc. the third day. Except for an increased urine excretion, no disturbance was observed; the urine was free from albumen. In experimenting with cattle, Moussu (*30*) used the pure oil of allyl isothiocyanate as well as cakes containing crotonyl isothiocyanate. Concerning allyl isothiocyanate given internally, he concludes that it is very toxic and may cause in doses of 2 gm. per 100 kilos speedy death, with the symptoms of an acute inflammation of the intestines.

No injury was observed from feeding large and varying amounts of rape-seed cakes, either dry or mixed with water, though cows of different ages were fed with the cakes which, according to Brioux (*6*), contained over ½ per cent of crotonyl isothiocyanate. One old cow, not in milking condition, weighing about 400 kgm., was fed in four periods of five days each the amount of 1 kgm. of cake per day, increasing to 3 kgm. per day. Apparently no injury was caused, although the cow received in the final period about 17 gm. of crotonyl isothiocyanate in the form of a cake, yielding 0.57 per cent of this oil. Brioux, on the basis of Moussu's experiments, concludes that allyl isothiocyanate is six or seven times as toxic as crotonyl isothiocyanate. He pointed however, to the fact that Moussu fed the volatile oil of allyl isothiocyanate directly, while previous authors, using the mustard cake, could feed without causing injury to the animal amounts which contained decidedly larger quantities of volatile mustard oil. For other experiments with allyl mustard oil see Carlier (*9*).

BACTERICIDAL ACTION

The bactericidal effect, so strong in the case of allyl mustard oil and so essential for the keeping qualities of prepared mustard, as Kossowicz (*24, p. 329*) and others have pointed out, is lacking or very weak so far as crotonyl isothiocyanate is concerned. Stein made the following interesting experiments.

To a number of test tubes containing 10 cc. of raw milk a small amount (on point of knife) of liver of sulphur and also increasing but definite amounts of allyl isothiocyanate and crotonyl isothiocyanate, respectively, were added. A test paper, moistened with lead salt solution, was fastened in the opening of the tube. The opening was then closed with cotton, and the tubes were set aside at from 38° to 40° C. for 24 hours. The blackening of the lead paper, caused by the bacterial formation of hydrogen sulphid and subsequent formation of lead sulphid, indicated in which experiments bacterial activity was not inhibited by the addition of either of the volatile oils. His results are given in Table VI.

The far greater bactericidal effect of allyl mustard oil is clearly evident. Further interesting data on the bactericidal action of allyl mustard oil and the amounts which prevent the growth of bacteria or yeasts belonging to different species are reported by Kossowicz (*23, p. 149–161*).

TABLE VI.—*Bactericidal action of allyl isothiocyanate and crotonyl isothiocyanate*

Allyl mustard oil.		Crotonyl mustard oil.	
Number of drops.[1]	Intensity of blackening.	Number of drops.[1]	Intensity of blackening.
1/20	10	1/20	10
1/10	10	1/10	10
1/5	8	1/5	10
1/3	1	1/3	10
1/2	0	1/2	10
1	0	1	10
.....	.....	2	10
.....	.....	3	5
.....	.....	4	5
.....	.....	6	3
.....	.....	8	2
.....	.....	10	0

[1] One drop=0.05 cc.

Certain manufacturers of prepared mustard who unwittingly used the Chinese colza seed as mustard in the usual proportions in preparing their product noted extensive spoilage within a short time. The deficiency of crotonyl isothiocyanate with respect to its bactericidal action is thus also demonstrated in a practical and very impressive way.

PHYSIOLOGICAL CONCLUSIONS

Crotonyl isothiocyanate differs distinctly from true volatile mustard oil (allyl isothiocyanate). Its flavor resembles that of cabbage or turnip instead of that of onion. No appreciable effect on the eye or the mucous membrane of the nose and no blistering effect on the skin were noted, in contrast to such and other effects of allyl isothiocyanate. Crotony isothiocyanate lacks also the pronounced bactericidal qualities of true volatile mustard oil. Moreover, crotonyl isothiocyanate is distinctly different from the nonvolatile mustard oil, para-oxybenzyl isothiocyanate, of white mustard, which has no odor but has a strong biting taste and a strong blistering effect on the skin.

SUMMARY

Material imported as rape seed and sold as mustard seed was identified as Chinese colza, *Brassica campestris chinoleifera* Viehoever, n. var.

The characteristics of the seed have been established, and those which permit the identification and differentiation from true mustard seed have been pointed out.

Plants have been grown from the seed, and the characteristics have been established, especially with reference to their close relationship to the colza group, *Brassica campestris*.

The volatile oil obtained from the material was identified as crotonyl isothiocyanate, which is not a suitable substitute for mustard oil, in respect to condimental, bactericidal, or medicinal value.

The fixed oil proved to be of the general composition of the rape oils, and the quantity of the oil present, amounting to more than 40 per cent, characterized the seed as a very valuable oil seed.

On the basis of the general composition of the seed and the character of the volatile oil, it is suggested that the pressed oil cake may well be used as a stock feed.

The leaves are succulent and should be of value as greens.

The plants, which are very vigorous and apparently hardy, seem to offer possibilities as a forage crop. Experiments along this line have been undertaken in cooperation with the Bureau of Plant Industry.

CONDENSED DESCRIPTION OF CHINESE COLZA (BRASSICA CAMPESTRIS CHINOLEIFERA VIEHOEVER)

Basal or radical leaves first single, later numerous, arranged in cluster, large glossy green, usually smooth, obovate or round, obovate in general outline, entire or obscurely wavy, variously toothed, sometimes crenate, tapering into a distinct thin petiole, which is more or less margined, showing sometimes a few leaflike lobes.

Leaves of flowering stem more or less glaucous, clasping, obovate, oblong, or somewhat lanceolate acuminate; leaves of secondary stem not always clasping.

Flowers light yellow, of medium size (generally that of mustard flowers), pedicels averaging well over ½ inch.

Pods rather large and long, tapering into conical beak (0.4 to 0.8 inch long); pod and beak together from 2 to 3 inches long; from 8 to 12 seeds in pod.

Seeds yellow and brown, yellow greatly predominating; somewhat compressed, oval, usually distinct ridge on ventral side, usually smooth brown, slightly reticulated, varying in size (from 1.69 to 2.07 mm.), weighing from 1.4 to 6.4 mgm. (1,000 seeds weighed 2.865 gm. and 500 cc. weighed 352 gm.).

SEED COAT.—Epidermis about 5 microns thick; when it is moistened shows no swelling, no polarization of light, or cell structure. Parenchyma almost completely compressed. Sclerenchyma (palisade cells), from 15 to 31 microns high and from 8 to 15 wide, strongly thickened at base and side, without pigment, inner wall smooth. Pigment layer consists of one row of cells containing pigment only in brown seeds. Protein layer is formed usually by one row of large cells (from 15 to 21 microns high and from 15 to 42 microns wide).

SEED.—Composition averages as follows: Over 40 per cent fatty oil (colza or rape oil type); about 23 per cent protein (N = 6.25); 11.5 per cent reducing substances; 4 per cent crude fiber; 0.5 per cent by hydrolysis of volatile oil consisting of crotonyl isothiocyanate.

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PLATE 10

A.—Yellow seed of Chinese colza. Approximately × 5.

B.—Brown seed of Chinese colza. Approximately × 5.

C.—Surface section of yellow seed of Chinese colza, showing lack of reticulations. Approximately × 103.

D.—Surface section of brown seed of Chinese colza, showing reticulations. Approximately × 103.

E.—Cross section of yellow seed of Chinese colza. Approximately × 289.

F.—Cross section of brown seed of Chinese colza. Approximately × 289.

E and F show the following:

(a) Tangentially striated epidermis.

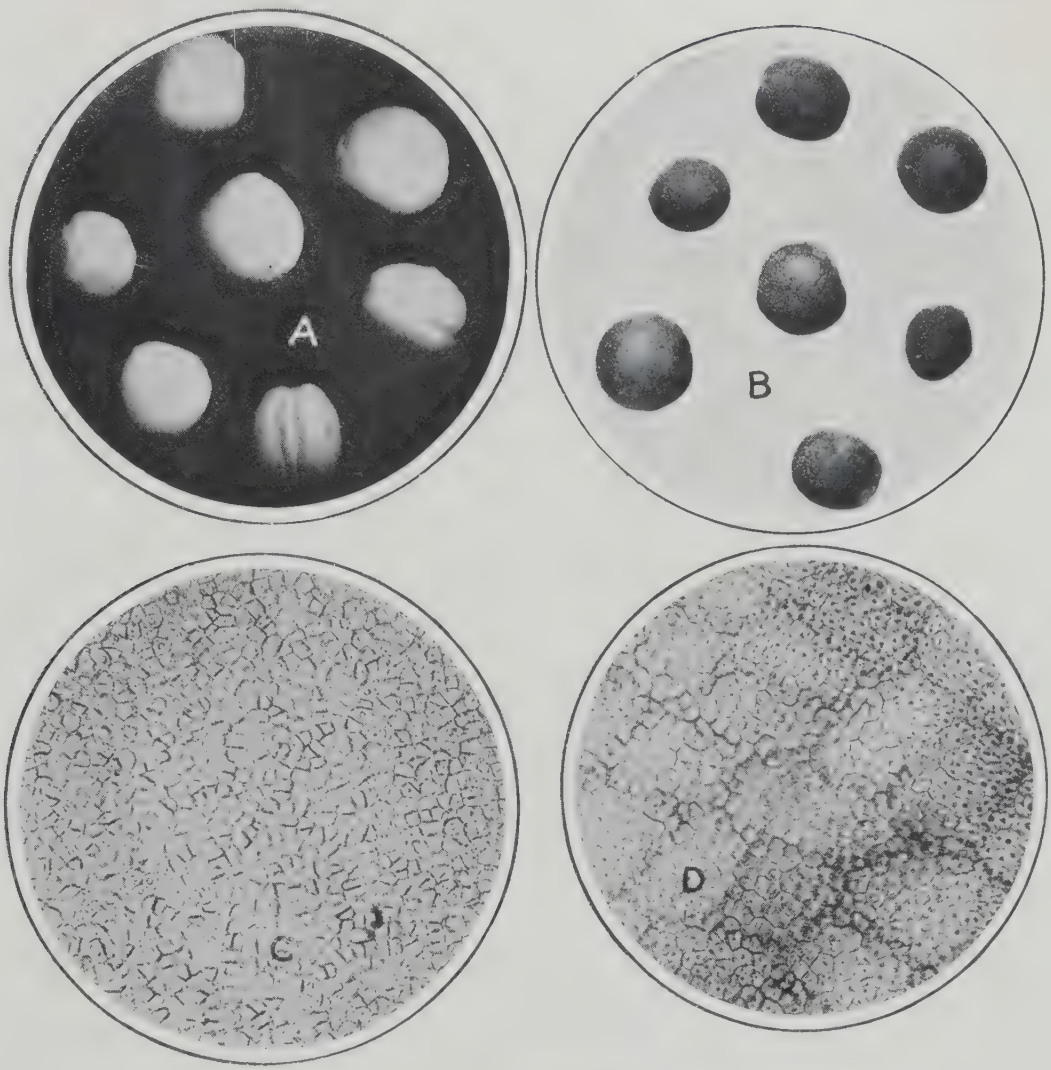
(b) Almost obliterated parenchyma.

(c) Sclerenchymatic palisade cells.

(d) Parenchyma, which in the brown seed (F) contains a pigment.

(e) Protein layer of the endosperm.

(f) Compressed parenchyma of the endosperm.


A
B
C
D


E
a
b
c
d
e
f
F
a
b
c
d
e
f


0 1 2 3 4 5
Centimeters

PLATE 11

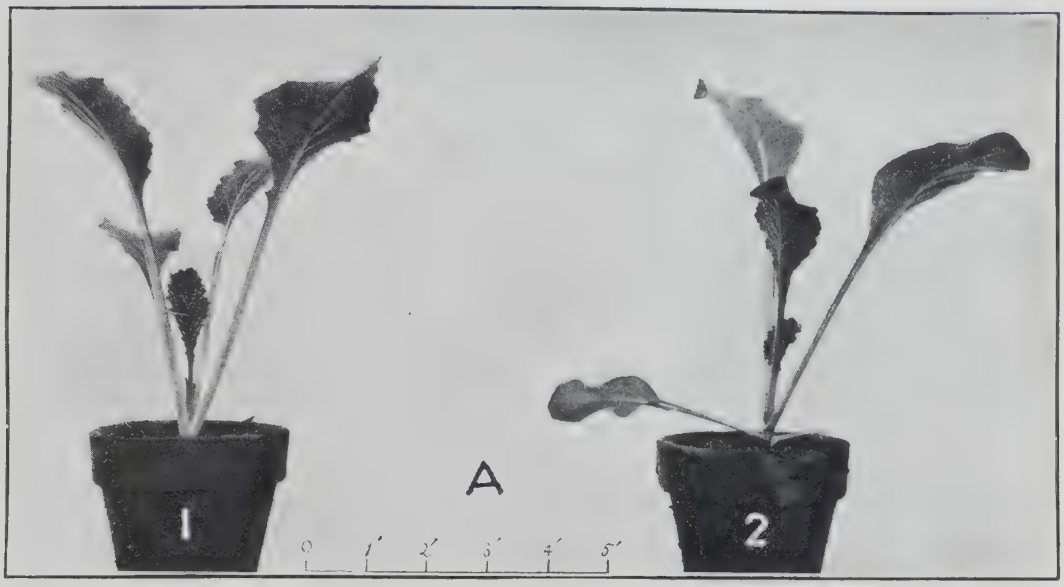
Seedling of Chinese colza, showing cotyledons and young leaves. The leaves show hairs, especially on the margin. Twenty-three days old. Grown in greenhouse. Planted March 23, 1917.

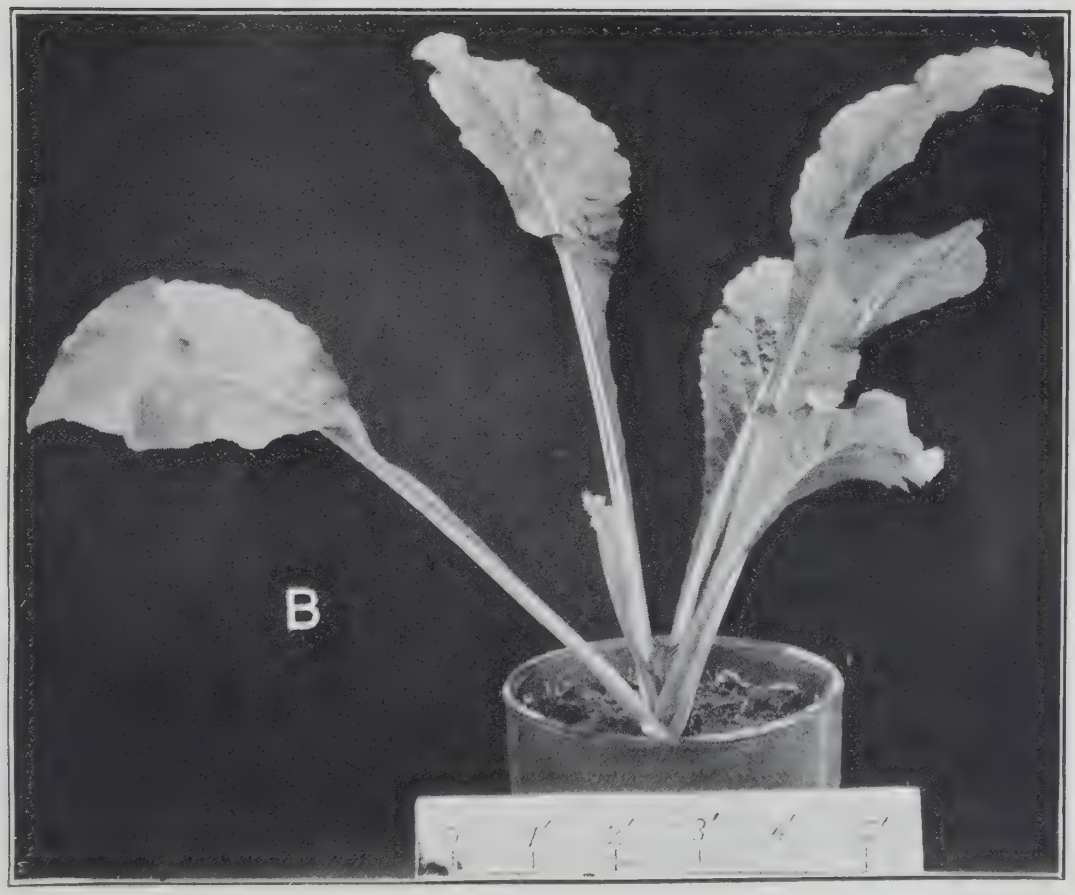
PLATE 12

Early rosette stage of Chinese colza seedling:

A.—Plants from (1) brown seed and (2) yellow seeds. Three weeks old. Grown in greenhouse. Planted March 14, 1917.

B.—Usual form, showing almost entire leaves. Three months old. Grown in greenhouse. Planted January 20, 1917.


A
1
2
0 1′ 2′ 3′ 4′ 5′


B
1′ 2′ 3′ 4′


A
0
1'
2'
3'
4
5'

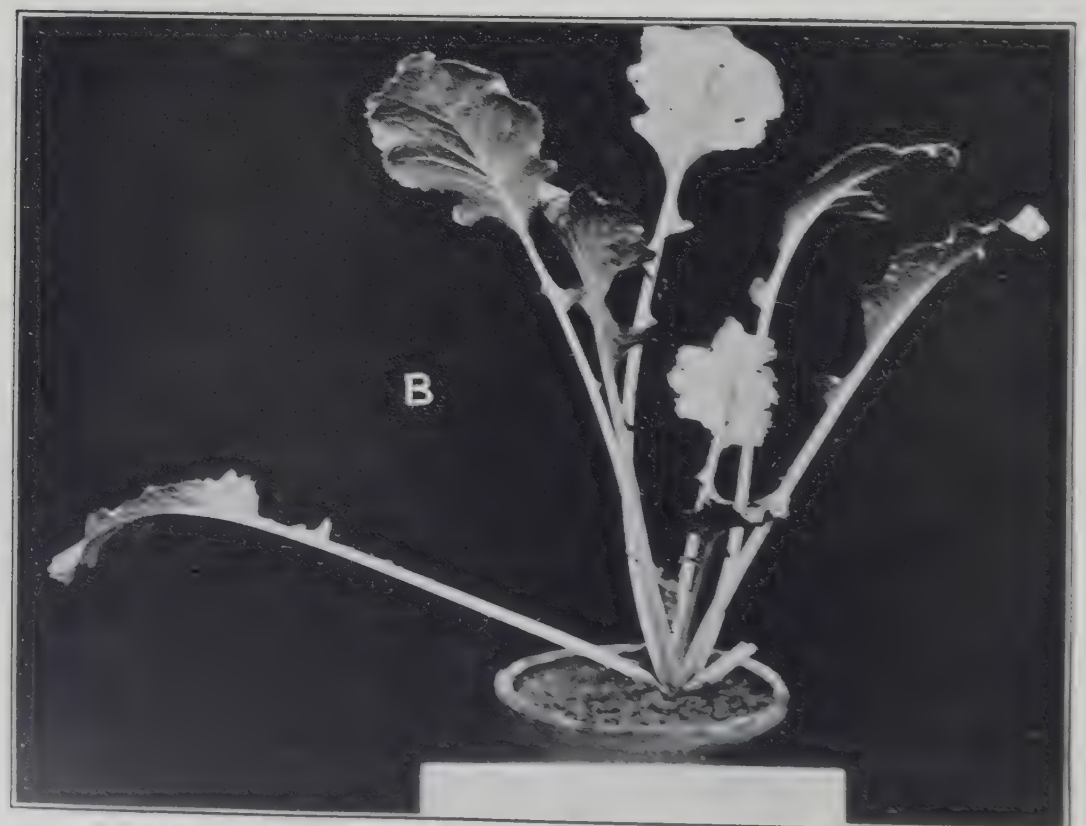

B

PLATE 13

Early rosette stage of Chinese colza seedling:

A.—Plant showing a variation in lobing of the leaves. Two months old. Grown in greenhouse. Planted February 20, 1917.

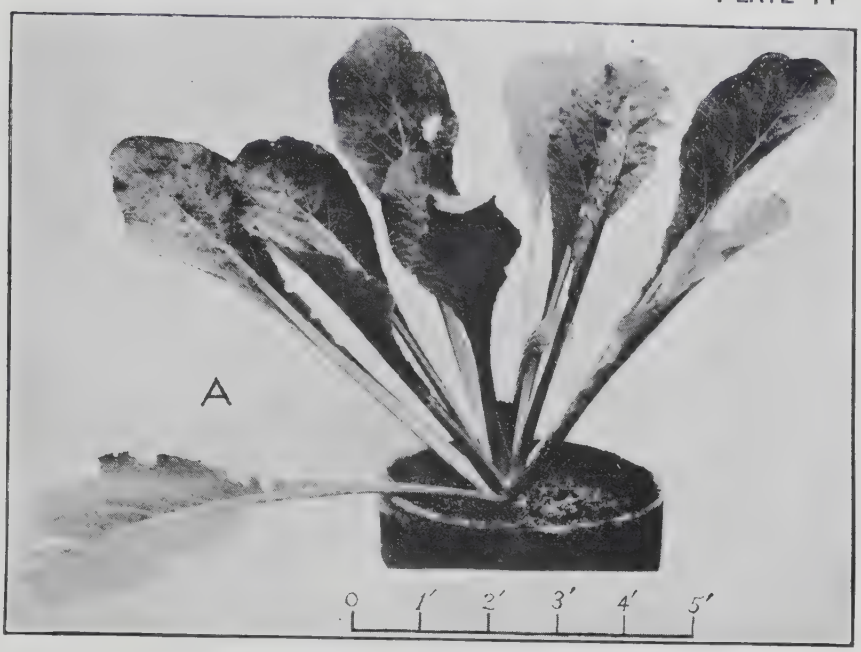
B.—Plant showing a variation in lobing of the leaves. Three months old. Grown in greenhouse. Planted February 20, 1917.

PLATE 14

Late rosette stage of Chinese colza seedling:

A.—Usual form. Three and one-half months old. Grown in greenhouse. Planted September 27, 1916.

B.—Plant showing a variation in lobing of the leaves. Two months old. Grown in field at Yarrow Station, Md. Planted May 16, 1917.


A
0 1′ 2′ 3′ 4′ 5′


B
0 1′ 2′ 3′ 4′ 5′ 6′


A


B


C

PLATE 15

Late rosette stage of Chinese colza seedling:

A.—Pe-tsai. Grown in field at Arlington, Va., by D. N. Shoemaker. The rule is 17¼ inches in length.

B.—Cross between Pak-choi and Pe-tsai. Grown in field at Arlington, Va., by D. N. Shoemaker. The rule is 15 inches in length.

C.—Pak-choi. Grown in field at Arlington, Va., by D. N. Shoemaker. The marked portion of the rule is 12 inches in length.

PLATE 16

Early flowering stage of Chinese colza:

A.—Usual form, showing somewhat enlarged stem base and stem-clasping leaves. Almost 5 months old. Grown in greenhouse. Planted September 27, 1916.

B.—Plant without enlarged stem base. Almost 5 months old. Grown in greenhouse. Planted February 20, 1917. The rule is 5 cm. in length.

C.—Usual form, showing glaucous leaves. Two months old. Grown in field at Arlington, Va. Planted about May 1, 1916.


A


B


C


A
0 1 2 3 4 5 6
B

PLATE 17

Early flowering stage of Chinese colza:

A.—Usual form, showing luxuriant growth and long pedicels. Two and one-half months old. Grown in field at Yarrow Station, Md. Planted May 16, 1917.

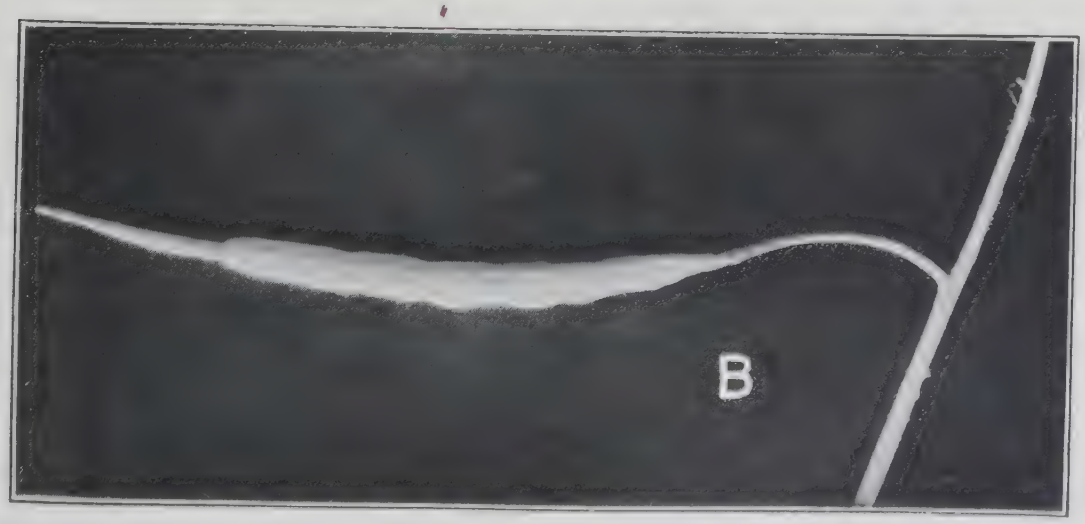
B.—Flower cluster. Plant about 5 months old. Grown in greenhouse. Planted October 4, 1916. Natural size.

PLATE 18

A.—Fruiting stage of Chinese colza. Plant about 3 months old. Grown in field at Arlington, Va. Planted May 1, 1916.

B.—Mature fruit of Chinese colza. From a plant 7 months old. Grown in greenhouse. Planted October 4, 1916. Natural size.


A


B


B


A

PLATE 19

A.—Herbarium specimen of *Brassica chinensis* L. Approximately × 1/5.
B.—Herbarium specimen of *Brassica campestris*. Approximately × 1/5.

STUDY OF SOME POULTRY FEED MIXTURES WITH REFERENCE TO THEIR POTENTIAL ACIDITY AND THEIR POTENTIAL ALKALINITY: I[1]

By B. F. KAUPP, *Investigator and Pathologist*, and J. E. IVEY, *Assistant in Poultry Husbandry, Research Laboratory of the Office of Poultry Investigations and Pathology, North Carolina Agricultural Experiment Station*

HISTORICAL REVIEW

Interest in the acid-base balance of dietaries has increased greatly in recent years. Sherman and his collaborators pointed out the basis for work of this kind when they made more accurate determinations than had hitherto been available of the ash constituents of the common feeds.

Sherman[2] has shown that meats and cereals have a preponderance of acid-forming elements, whereas, on the other hand, fruits and vegetables have an excess of base-forming elements.

It has been shown that ash has an influence on the reaction of the urine. Acid-forming feeds lead to the formation of more acid urines, and base-forming feeds cause the excretion of less acid or of alkaline urines. However, it has been found in studies carried on with men that certain exceptions were found—namely, plums, prunes, and cranberries, which, although yielding a basic ash, nevertheless increase the acid excretion because of the presence of benzoic acid, excreted as hippuric acid.[3]

Although the question whether or not an acid-forming diet eaten for some period of time is productive of undesirable results is debatable, probably the concensus of opinion is in favor of diets in which the acid-forming and base-forming elements are approximately balanced. The possibility that the continued use of acid-forming diets may lead to a greater susceptibility to disease of the less infectious type has seemed worthy of investigation.[4] Work along this line is in progress in this laboratory. Sour milk and buttermilk function as base because when used by the body the lactic acid is oxidized to carbonic acid, which is thrown off by the lungs, leaving a base residue of mineral salts. A common defect is the use of quantities of proteins and fats far in excess of the needs of the body. Proteins and fats are relatively expensive materials.

[1] This paper deals with the flocks for the first 24 weeks. Part II will deal with the first laying year.

[2] SHERMAN, Henry C. FOOD PRODUCTS. ix, 594 p., illus. New York, 1919.

[3] BLATHERWICK, N. R. THE SPECIFIC RÔLE OF FOODS IN RELATION TO THE COMPOSITION OF THE URINE. *In* Arch. Int. Med., v. 14, p. 409–450. 1914.

[4] HINDHEDE, M. PROTEIN AND NUTRITION . . . p. 8. London, 1913.

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Key No. N. C.-14

THE PROBLEM

Profitable production of broilers begins with the baby chicks and extends over a period of about eight weeks, at the end of which time the birds should weigh, as a flock, approximately 1½ pounds each.

Our problem consisted of two parts:

1. To ascertain the mineral content of poultry feeds and, from this as a basis, to determine the potential acidity and potential alkalinity of these feeds.

2. To determine the acid-base balance of the feed mixtures used in our experimental feeding work in the production of broilers, giving some of the feeding results.

EXPERIMENTAL METHODS

The baby chicks were produced from a single flock of pure-bred Single-Comb White Leghorns, bred at the station and college poultry plant, and were hatched in the same incubator. Each lot was housed under similar oil-burning hovers of 100-chick capacity.

The experiment was carried on in periods of 8 weeks each and extended over three periods, or 24 weeks. The samples of feeds for analyses were obtained, as composite samples, from the various bags of feed used in the experiment. The potential acidity and potential alkalinity were estimated from the mineral analyses of the feed samples.

The caloric values of all the animal food rations—No. 2, 3, 4, and 7—are about the same, being 27 to 31 per cent protein calories. The soybean meal ration is in the same column so far as protein calories are concerned. The grain feeds have less protein caloric value, having only 12 per cent. Dried buttermilk functions as base. The meat scrap and bone meal and the digester tankage are base on account of the large amount of calcium in the bone. Blood is normally alkaline because of the sodium salts. The grain ration is 61.96 cc. acid per pound. The mashes are all alkaline or base. Those containing milk or bone—rations 2, 3, and 4—run high in base elements, while the blood meal runs only 41.21 cc. base and compares favorably with the two rations containing no animal food but containing protein from leguminous sources.

It will be noted that ration No. 6, the peanut-meal mixture, is low in protein caloric value. This is due to the fact that the peanut meal used was ground peanuts and hulls, not fat-extracted, and showed 40.4 per cent fat content.

Many interesting things are brought out by Table IV. The greatest gain in weight in chicks is during the first eight weeks, or the first period. Following the first period the increase in weight is gradually less during the remainder of the two periods.

The amount of feed required to produce a pound of gain gradually grows greater as the bird becomes older.

TABLE I.—*Mineral content of southern poultry feeds* [a]

[Results expressed in parts per hundred]

Kinds of feed.	Number of analyses.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
Corn meal, bolted	4	0.349	0.072	0.0092	0.1336	0.160	0.0244	0.341	0.0040
Pinhead oats	7	.441	.109	.0126	.0704	.236	.0900	.499	.0019
Whole wheat	13	.435	.039	.0271	.1127	.183	.0630	.436	.0070
Wheat middlings	6	.949	1.219	.0980	.3628	.232	.0603	.783	.0052
Bone meal	3	.229	.735	21.1770	.5800	.170	.0900	10.349	.0180
Meat and bone meal	8	.158	.473	13.2927	.8333	.238	.5883	5.029	.1252
Rolled oats	2	.370	.136	.0430	.1560	.256	.0238	.473	.0062
Whole corn	13	.332	.041	.0127	.1051	.148	.0521	.293	.0044
Hulled oats	6	.387	.053	.0915	.1465	.204	.0870	.454	.1090
Velvet bean meal [b]	1	1.186	.141	.3600	.2080	.151	.2220	.764	.0126
Soybean meal	5	1.179	.422	.2901	.2503	.330	.0373	.481	.0241
Peanut meal	5	1.191	.422	.2401	.2106	.325	.0312	.567	.0271
Skim milk	2	.151	.144	.1530	.0019	.042	.0650	.136	.0036
Egg, including shell	3	.0103	.200	.6080	.0985	.395	.1500	.3021	.0103
Rape, green	3	.2510	.008	.0084	.0206	.035	.0930	.0102	.00000076
Limestone grit	2	.0000	.000	30.9700	6.6700	.000	.0000	.0000	3.3330
Oyster shell	2	.0000	.000	37.9510	.4200	.147	.0900	.0000	.37500
Dried buttermilk	4	.7084	.9531	1.3491	.8107	.084	.3650	.1691	.4301
Digester tankage	4	.3605	.1948	3.0147	1.1654	.124	.3483	1.2323	.10175
Dried blood	4	.0941	.3223	.3755	.2175	.281	.1381	.2864	.01650

[a] Average of all analyses to Mar. 1, 1920.

[b] Velvet bean meal and pod.

TABLE II.—*Potential acidity and potential alkalinity of southern poultry feeds*

Kinds of feed.	Base.[a]	Acid.[a]
Corn meal, bolted	.....	21.75
Pinhead oats	.....	121.94
Whole wheat	.....	79.09
Wheat middlings	206.58	.....
Bone meal	2,104.60	.....
Meat and bone meal	565.13	.....
Rolled oats	.....	108.21
Whole corn	.....	44.76
Hulled oats	.....	79.17
Velvet bean meal	62.17	.....
Soybean meal	156.01	.....
Peanut meal	94.90	.....
Skim milk	23.18	.....
Rape, green	15.33	.....
Limestone grit	11,943.80	.....
Oyster shell	8,782.10	.....
Dried buttermilk	803.96	.....
Digester tankage	789.89	.....
Dried blood	63.68	.....
Egg, including shell	.....	2.55
Peas, dried	24.00	.....
Potatoes, sweet	24.00	.....
Potatoes, Irish	26.00	.....
Rice	.....	43.00
Spinach	122.00	.....
Turnips	25.00	.....
Beans, dried	79.00	.....
Beets	50.00	.....
Bread, hard	.....	38.00
Cabbage	22.00	.....
Carrots	38.00	.....
Fish, dried	.....	36.00
Hominy	.....	24.00
Lettuce	27.00	.....

[a] Expressed in excess cubic centimeters per pound of feed.

The bird gradually, in these tests, consumed more grain as it grew older. Likewise it was found that as a pullet comes into laying it consumes a greater proportion of mash and again slackens in its mash consumption as it goes into a nonlaying period.

The consumption of more grain and less mash has a tendency to lessen the base balance or, if the balance is already acid, has a tendency to increase the acid balance.

It was noted that the cereals are decidedly acid while the by-products from the legumes are of a base reaction. Feeds containing by-products, such as soybean meal and peanut meal, have a tendency to add to the base balance.

It is noted that rations containing buttermilk, digester tankage, and meat scrap and bone meal give a base balance in all cases.

The soda in the blood makes the blood meal base but not so strongly so as digester tankage or meat and bone meal containing much bone.

Bone is rich in calcium and also contains other bases such as sodium. As stated before, sour milk functions as base.

TABLE III.—*Acid-base balance of rations 1 to 7*

Ration No.	Kinds of feed.	Amount.	Acid.[a]	Base.[a]	Percentage of protein calories.
		Pounds.			
1	Scratch feed:				
	Corn	100	.....	.....	.....
	Oats	100	61.96	.....	12
	Mash feed:				
2	Wheat middlings	35	.....	.....	.....
	Corn meal	30	.....	.....	.....
	Ground oats	35	.....	.....	.....
	Dried buttermilk	35	.....	231.4	27
3	Wheat middlings	35	.....	.....	.....
	Corn meal	30	.....	.....	.....
	Ground oats	35	.....	.....	.....
	Meat and bone meal	20	.....	125.9	30
4	Wheat middlings	35	.....	.....	.....
	Corn meal	30	.....	.....	.....
	Ground oats	35	.....	.....	.....
	Digester tankage	18	.....	153.7	30
5	Wheat middlings	35	.....	.....	.....
	Corn meal	30	.....	.....	.....
	Ground oats	35	.....	.....	.....
	Soybean meal	24	.....	63.9	30
6	Wheat middlings	35	.....	.....	.....
	Corn meal	30	.....	.....	.....
	Ground oats	35	.....	.....	.....
	Peanut meal	41	.....	54.5	19
7	Wheat middlings	35	.....	.....	.....
	Corn meal	30	.....	.....	.....
	Ground oats	35	.....	.....	.....
	Blood meal	14	.....	41.21	31

[a] Expressed in excess cubic centimeters per pound of feed.

In this work no account has been taken of the amount of calcium entering the crop as grit and oyster shell. This will, in all probability, overcome the acid reaction, though without definite data this is a mere guess.

In a large table of studies of rations furnished by the medical staff of the United States Army, the percentage of protein calories ran from 10 to 18. In the present work the percentage of protein calories ran from 13 to 22, according to the estimate of the actual intake in each period. There is a possibility, however, that we will need to pay more attention to the source or kinds and quantities of protein calories, since we have shown in this work that those birds that received animal food, including milk, tankage, meat and bone meal, and dried blood were prepared by the storing up of the proper potential energy to begin heavy egg production very young, while those birds that did not receive animal food of any kind were not prepared and did not go into early heavy egg production.

TABLE IV.—*Relation of protein calories, amount of feed, and acid-base balance to pound of gain with rations 1 to 7*

Ration No.	Period.[a]	Amount of feed consumed.			Amount of feed per pound of gain.	Percentage of gain.	Excess acid.[b]	Excess base.[b]	Percentage of protein calories.	Test protein.
		Mash.	Grain.	Total.						
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*					
1 and 2	First	23.3	15.4	38.7	3.0	[c] 800	.....	114.6	21	Buttermilk.
	Second	35.2	28.6	63.8	[d] 4.2	200	.....	99.8	20	
	Third	30.3	54.5	84.8	[d] 6.0	63	.....	42.8	17	
1 and 3	First	22.8	18.7	41.5	2.7	905	.....	41.2	21	Meat and bone meal.
	Second	36.7	35.3	72.0	4.9	137	.....	33.8	21	
	Third	34.8	61.9	96.7	4.9	50	.....	5.6	17	
1 and 4	First	23.6	20.0	43.6	1.8	1,142	.....	54.8	21	Digester tankage.
	Second	17.5	23.7	41.2	6.4	141	.....	29.6	19	
	Third	14.3	22.2	36.5	6.0	62	.....	22.5	19	
1 and 7	First	19.7	20.8	40.5	2.1	1,300	11.7	.....	21	Blood meal.
	Second	33.8	33.1	66.9	5.8	83	9.8	.....	21	
	Third	26.1	48.7	74.8	7.0	49	25.8	.....	18	
1 and 5	First	19.5	14.5	34.0	2.9	900	.....	10.2	22	Soybean meal.
	Second	31.3	36.9	68.2	5.9	133	4.1	.....	20	
	Third	30.0	56.9	86.9	8.4	51	18.4	.....	18	
1 and 6	First	32.7	21.9	54.6	3.1	800	.....	7.8	16	Peanut meal.
	Second	30.9	27.3	58.2	4.7	206	.1	.....	15	
	Third	10.4	48.4	58.8	5.3	59	41.3	.....	13	

[a] Each period consists of eight weeks.
[b] Expressed in excess cubic centimeters per pound of feed.
[c] Computed on the basis of weight of the baby chicks at hatching and at the beginning of each period.
[d] In the latter part of the third period, in all the flocks receiving animal food, egg production began. As egg production begins it is noted that the birds consume relatively more mash, which has a tendency to lessen the acid balance. If the birds are not in egg production, less mash and more grain are consumed, which increases the acid balance.

TABLE V.—*Acid-base balance of rations 8 to 11*

Ration No.	Kinds of feed.	Amount.	Acid.[a]	Base.[a]	Percentage of protein calories.
		Pounds.			
8	Rolled oats	8	.....	.....	.....
	Wheat middlings	8	.....	.....	.....
	Meat and bone meal	2	.....	.....	.....
	Bone meal	1	.....	128.3	29
9	Cracked wheat	3	.....	.....	.....
	Cracked corn	2	.....	.....	.....
	Pinhead oats	1	67.2	.....	13
10	Wheat middlings	6	.....	.....	.....
	Corn meal	3	.....	.....	.....
	Meat and bone meal	3	.....	.....	.....
	Bone meal	1	.....	382.6	30
11	Whole wheat	3	.....	.....	.....
	Cracked corn	2	.....	.....	.....
	Hulled oats	1	67.2	.....	13

[a] Expressed in excess cubic centimeters per pound of gain.

TABLE VI.—*Relation of protein calories, amount of feed, and acid-base balance, to pound of gain during first eight weeks*

Kinds of feed.	Amount.	Excess acid.[a]	Excess base.[a]
	Pounds.		
Milk	52.30	.....	1,212.31
Ration No. 8	2.42	.....	310.48
Ration No. 9	8.42	565.82	.....
Ration No. 10	4.30	.....	1,645.18
Ration No. 11	5.98	401.85	.....
Rape	7.81	.....	119.72

[a] Expressed in excess cubic centimeters in each feed consumed.

Amount of feed per pound of gain, 3.4 pounds.
Percentage of gain, 686.
Excess base intake, 2,320 cc.
Excess base intake per pound of feed, 85.5 cc.

SUMMARY

From Table II we note that corn, wheat, and oats, as well as egg with the shell, rice, bread, fish, and hominy are acid. The wheat middlings, in six analyses, on account of the high content of sodium and potassium, are base. Green feeds, such as rape, cabbage, carrots, beets, turnips, potatoes, spinach, and lettuce, are base. Seeds of the legumes, such as velvet bean, soybean, peanut, and peas, are base. Bone meal, on account of its high calcium content as well as other base-forming elements, is highly base. Limestone grit is very highly base, and also to a less extent is crushed oyster shell. The animal feeds containing bone, such as meat and bone meal and digester tankage, are base. The calcium of the egg shell does not quite overcome the acid of the albumin of the egg. Dried

milk functions as base because the lactic acid is oxidized to carbonic acid, which is thrown off by the lungs, leaving the basic residue of mineral salts. Dried skim milk or dried buttermilk is therefore quite base in function. Dried blood, on account of its magnesium, calcium, and sodium content, is moderately base.

In these studies there have been arranged 11 feed mixtures for acid-base studies. The first 7 are North Carolina Experiment Station formulae and the last 4 are those of Prof. Rice. The mixtures that contain considerable amounts of either dried milk, meat and bone meal, or digester tankage are quite base. The mixture containing soybean meal is approximately as much base as the grain mixture is acid, so that equal amounts would approximately balance so far as acid-base content is concerned. The peanut meal mixture is slightly below the soybean meal mixture, and the blood meal comes slightly below the peanut meal.

We note from Table III that the grain mixture contains 12 per cent protein calories and the ground feed mixtures contain from 19 per cent in the mixture containing peanut meal in which peanut meal not fat-extracted to 31 per cent in the ration in which blood meal was used. We note by a study of Table IV, which gives the total intake of each mixture for each period, that the final percentage of protein calories runs from 13 to 22. For comparison with rations for human beings we may again refer to the study of army rations during the late World War, in which the percentage of protein calories ran from 10 to 18. Dr. Osborne[1] found that 12.5 per cent protein calories produced maximum growth in rats. The indications are that the kinds as well as the quantities of proteins are essential factors. While the kinds of amino acids and vitamins are important factors in addition to kinds and quantities of minerals, there is a possibility that there are other factors undiscovered which have a profound bearing on growth, egg production, and the preparing of pullets, by aiding the storing up of potential energy, for early and heavy egg production. Data which will be published later show that pullets grown on range or in confinement without animal food of any kind, though the protein calories were above those indicated in comparison rations,[1] were not prepared for early heavy egg production and did not show high egg yields until animal food of some kind had been added. In this instance this was the soybean meal and peanut-meal lots. In the second and third periods the balances of intake was acid.

Further studies are being made to determine whether acid feeds will in any way interfere with either growth or egg production. In these studies rations 5 and 6 can be made base by the addition of ground limestone or gound oyster shell. The amounts to be added would depend upon the proportions in which the mash and grain were fed.

[1] OSBORNE, Thomas B., and MENDEL, Lafayette B. A QUANTITATIVE COMPARISON OF CASEIN, LACTALBUMIN, AND EDESTIN FOR GROWTH OR MAINTENANCE. *In* Jour. Biol. Chem., v. 26, no. 1, p. 3. 1916.

We find, by a study of Table V, that the grain rations 9 and 11 are acid and that the mash is base. In these mash mixtures there has been added both bone meal and meat and bone meal. Wheat middlings also aid in overcoming the acidity of corn meal and of rolled oats. In this test the total intake excess was base. The percentage of protein calories was 22.

CONCLUSIONS

Grain mixtures as ordinarily used in poultry feeding are acid.

Mash mixtures containing sufficient quantities of digester tankage, meat and bone meal, dried milk, or dried blood will be base.

Acid balances of feed mixtures can be overcome by the addition to mashes of dried milk, digester tankage, meat and bone meal, bone meal, dried blood, or ground limestone or oyster shell. Green feed, milk to drink, and limestone and oyster-shell grit also aid in overcoming the acid balance of grain mixtures.

THE INFLUENCE OF COLD IN STIMULATING THE GROWTH OF PLANTS [1]

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In regions having a cold winter like ours, with prolonged or repeated freezing, the native trees and shrubs become dormant in autumn. According to the general belief this condition is brought about by the cold. It is also the general belief that warm weather is of itself the sufficient cause of the beginning of new growth in spring. Both these ideas are erroneous. It is the object of the present address to show: first, that in our native trees and shrubs dormancy sets in before cold weather, and that cold weather is not necessary for the establishment of complete dormancy; second, that after such dormancy has begun, the exposure of the plants to an ordinary growing temperature does not suffice to start them into growth; third, that these plants will not resume normal growth in the warm weather of spring unless they have been subjected previously to a period of chilling; and, finally, a theory will be advanced to explain this paradoxical effect of cold in stimulating growth instead of retarding it.

The subject will be presented in a series of numbered statements, each followed by supporting evidence.

1. TREES AND SHRUBS OF COLD CLIMATES BECOME DORMANT AT THE END OF THE GROWING SEASON WITHOUT THE NECESSITY OF EXPOSURE TO COLD WEATHER.

A little more than 10 years ago, while engaged in a series of greenhouse experiments, the speaker came upon a strange phenomenon which was wholly unexpected and which threatened to interfere seriously with the success of the experiments. Healthy blueberry plants, intended to be used during the winter for breeding purposes, were brought into the greenhouse at the end of summer and were kept at an ordinary growing temperature. They refused to continue their growth during the autumn, gradually dropped their leaves, and went into a condition of complete dormancy. They did this at a greenhouse temperature which in spring and summer would have kept the plants in a condition of luxuriant growth. The completeness of the condition of dormancy which such plants reach can be best appreciated from photographs (Pl. 20, A).

Since 1910 this experiment has been repeated many times and with many species of plants, and without exception those trees and shrubs

[1] An address delivered before the National Academy of Sciences Apr. 27, 1920.

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native of our northern cold-winter region which were tested went dormant in fall or winter regardless of temperature. In comparing outdoor plants with indoor plants of the same species the most that can be said in favor of outdoor conditions is that dormancy progresses a little faster in outdoor plants, evidently because their foliage is injured by freezing weather, and they drop their leaves somewhat earlier than indoor plants.

2. TREES AND SHRUBS THAT ARE KEPT CONTINUOUSLY WARM DURING THE WINTER START INTO GROWTH MUCH LATER IN SPRING THAN THOSE THAT HAVE BEEN SUBJECTED TO A PERIOD OF CHILLING.

In the late winter and early spring of 1910 I waited patiently, and then impatiently, for my indoor plants to bloom, and at last I was forced to realize that they never would bloom. When compared with plants of the same kind that had been outdoors during the winter and had been brought into the greenhouse in early spring, the difference was astonishing. The outdoor plants burst into leaf and flower luxuriantly, while the indoor plants remained completely dormant and naked. The experiment was repeated many times and with various species of plants, some of which may be used in illustration. (See Pl. 20, B; 21; 22, A.)

At first it was supposed that the plants needed to be frozen to start them into growth, but a single freezing proved not to be effective. And then it was found that the dormant plants would start into growth without any freezing whatever. It was necessary only that they be subjected to a period of prolonged chilling, usually two or three months, at a temperature a few degrees above freezing.

If plants are kept continuously in a warm place without chilling, the dormant condition often continues for an extraordinary length of time. In some instances plants have remained dormant for a whole year under conditions of heat, light, and moisture that ordinarily would make the same plant grow with the greatest luxuriance.

3. THE STIMULATING EFFECT OF COLD IS LIMITED TO SUCH PORTIONS OF THE PLANT AS ARE SUBJECTED TO THE CHILLING.

The conspicuous difference in spring growth between chilled plants and plants not chilled has already been shown. These differences, furthermore, can be produced experimentally upon different parts of the same plant. Plants thus treated present a very curious and remarkable appearance, as shown in Plate 22, B, and Plate 23.

On February 3, 1912, a blueberry plant (Pl. 22, B) 44 inches in height, which had shed its leaves and become dormant in a warm greenhouse maintained at a temperature of 60° to 70° F., was subjected to the following experiment: It was repotted in a 7-inch pot and set in the south end of a greenhouse at the temperature already mentioned. A small opening was made in the glass, and through this opening one of the two stems of the plant was pushed. The open space about the stem where it passed through the glass was carefully plugged with moss. During

the rest of the winter the plant remained in the same position, the pot and the stem, shown at the left in the illustration, continuing in the warm temperature of the greenhouse, while the stem at the right, projecting through the glass, was exposed to the rigors of winter, with its alternate freezing and thawing. The illustration, from a photograph made April 18, shows that when spring came the outdoor branch started into normal growth while the indoor branch continued dormant.

A second illustration (Pl. 23) shows a modification of the first experiment. In this case the plant was set on a shelf outside the greenhouse, and a single branch was passed through the glass wall into the warm interior. When spring came it was this interior branch that remained dormant, all the outside branches putting out leaves promptly and normally.

From a comparison of the two experiments it is evident that the difference in behavior of the indoor and outdoor branches could not have been caused by any special action of the root system, for in one experiment the roots were inside, in the other outside. It is clear that the causes that stimulated growth in the exposed stems operated in the stem itself, not in the roots. This principle is still further exemplified and confirmed by the behavior of cuttings taken from blueberry plants in the first stages of their dormancy. Such cuttings if kept warm continue their dormancy into late spring or summer, but if chilled for two or three months they start into growth at the normal time in early spring.

It should be stated here that the difference in the amount of light inside and outside the greenhouse had nothing to do with the stimulation to growth, for chilled plants are ready to start into growth promptly whether the chilling is done in the full light of an outdoor situation, or in the partial light of a greenhouse, or in the complete darkness of an ordinary refrigerator.

4. THE STIMULATING EFFECT PRODUCED ON DORMANT PLANTS BY COLD IS INTIMATELY ASSOCIATED WITH THE TRANSFORMATION OF STORED STARCH INTO SUGAR.

In most of our wild species of trees and shrubs the reserve carbohydrate material is stored away during summer and autumn in the form of starch. At the beginning of dormancy the twigs and sapwood are gorged with this material, the starch grains being stored ordinarily in the cells of the medullary rays and sometimes in the pith. As the process of chilling goes on, this starch little by little is transformed into sugar. The presence of large quantities of starch in the fall and early winter may be observed by applying to freshly cut surfaces of the twigs the well-known starch test of a 2 per cent solution of iodin in a 1 per cent solution of iodid of potassium. With a strong hand lens the starch is readily observed, if present, by the deep blue color it assumes under this treatment. The intensity of the coloration gives roughly an idea of the

number of starch grains present, and thus by this simple means anyone may observe in the twigs of trees and shrubs the gradual disappearance of their starch as spring approaches.

The measurement of the increasing amount of sugar is more difficult and must be done by chemical analysis. Through the courtesy of the Chief of the Bureau of Chemistry, exact data can be presented on this point from analyses by Mr. Lorin H. Bailey. In samples of dormant blueberry wood taken in early spring when growth was about to begin the ratio of sugar to starch proved to be seven times what it was in similar dormant wood taken in autumn.

I desire at this time to comment on the fact that one of my colleagues reading the manuscript outline of this address criticized the use of the word "stimulate" as applied to the effect which chilling produces on these dormant plants. His idea was that the chilling induced certain physiological changes in the cell contents but that the actual stimulation to growth came from the temperatures that followed the chilling. I defend, however, the propriety of the language I have used, for although the later stages of growth admittedly can not take place without warm temperatures, not only does the transformation from starch to sugar take place at the chilling temperature but the buds actually swell and push if the chilling temperature is continued for several months. In illustration I may cite the following experiments.

On March 3, 1915, 286 cuttings were made from dormant outdoor blueberry plants. They were stored in bundles, some in moist sphagnum moss, others in moist birch sawdust, at a contemplated temperature of 31° F., just below freezing. The cuttings remained in cold storage until December 6, a little more than nine months. An examination of the cuttings on that date showed that one or more buds had begun to swell on every cutting with the exception of a small number which were mildewed and dead. In other words, growth had already begun to take place at the cold-storage temperature. The thermograph record for the 278 days was as follows:

	Hours.
29° to 32° F.	5,591
32° to 33° F.	990
33° to 34° F.	91

The temperature record did not go above 34° F. It is an astonishing fact that temperatures so very near freezing will start dormant plants into growth.

On March 3, 1915, 58 cuttings from dormant, outdoor blueberry plants were placed in moist birch sawdust in commercial cold storage at 33° to 36° F. On December 4, nine months later, buds on every cutting had begun to grow. Not one of these cuttings gave a starch reaction when tested with iodin. The transformation of their stored starch into sugar was complete. (See Pl. 24.)

5. THE THEORY ADVANCED IN EXPLANATION OF THE FORMATION OF SUGAR DURING THE PROCESS OF CHILLING IS THAT THE STARCH GRAINS STORED IN THE CELLS OF THE PLANT ARE AT FIRST SEPARATED BY THE LIVING AND ACTIVE CELL MEMBRANES FROM THE ENZYM THAT WOULD TRANSFORM THE STARCH INTO SUGAR, BUT WHEN THE PLANT IS CHILLED THE VITAL ACTIVITY OF THE CELL MEMBRANE IS WEAKENED SO THAT THE ENZYM "LEAKS" THROUGH IT, COMES IN CONTACT WITH THE STARCH, AND TURNS IT INTO SUGAR.

I have stated the theory in these words out of regard for simplicity and general understanding, but if anyone should require that it be presented in orthodox technical language it might be restated as follows: The reserve amylum carbohydrate bodies are isolated from the amylolytic enzym by semipermeable protoplasmic living membranes of high osmotic efficiency, but under the influence of low temperatures the protoplasmic membranes are proximately devitalized, they become permeable to the amylolytic enzym, and amylolysis ensues. I may add, however, that the use of such terminology seems to me to involve a certain degree of unnecessary cruelty.

From the evidence already presented, no one, presumably, will question that the chilling of dormant trees and shrubs is followed by growth and that the growth is associated with the transformation of starch into sugar. But the hypothesis that this transformation is brought about by the weakening of the cell membrane and the consequent leakage of starch-transforming enzyms into the starch chambers may very properly be challenged. In the Tropics there is no chilling weather, yet trees and shrubs spring into growth after the dormant period of the dry season just as they do in temperate climates after the dormant period of winter. The critical scientific man will therefore ask, "Are there not other agencies than chilling which will start dormant trees and shrubs into growth even in our latitude?" It must be said in reply that there are. And it will be worth while to consider some of these causes, for not only are they of interest in themselves but also, instead of weakening the hypothesis here presented, they serve to strengthen and confirm it.

The data may best be presented through a series of illustrations.

The pruning of a long-dormant plant will often start it into growth (Pl. 25, A). Girdling produces a similar result (Pl. 25, B, at left). Notching the stem does the same (Pl. 25, B, at right). Rubbing the stem also starts the plant into growth (Pl. 26).

In all these examples of the stimulation of growth by injury it is conceived that the enzym is brought into contact with the starch as a direct result of the breaking and straining of the cells. Sugar is then formed and growth begins.

It should be observed that when a normal chilled plant starts growing it grows from many buds (Pl. 27, A), for the effect of the chilling on sugar formation is general. When a dormant plant starts growing as the result

of injury, however, it usually starts, as shown in several illustrations already presented, from a single bud, the one nearest the point of injury. The injury is local, and both the sugar formation and the growth that follows it are local.

We are now brought to the consideration of a phenomenon which I take to be of special significance—namely, the procedure by which the dormant plant starts itself into growth in the absence of chilling. After a blueberry plant has remained dormant at a warm temperature for a very long period, sometimes a whole year, the tips of the naked branches begin to lose their vitality. Just before or just after the death of the tip a single bud, or sometimes two buds, situated next below the dead or dying part starts growing (see Pl. 27, B; 31, A). The new growth of the stem is confined to the one or two buds, just as it was in the case of growth induced by injury. My interpretation of the phenomenon is that, as death approaches, the cell membranes become weakened in much the same way as when chilled, the enzym passes through into the starch storage cells, sugar is formed, and the adjacent bud begins to grow. The process going forward here in a restricted portion of the stem, and due to a local cause, is essentially the same as that taking place generally over the plant, from a general cause, when the plant is chilled.

In the Tropics some plants are able to grow continuously; others become dormant in the dry season and start into growth again at the coming of the rainy season. Tropical plants probably have various methods of coming out of their dormancy, and there is every reason to expect that some of them will be found to accomplish this act in the same way as our long dormant greenhouse plants, by the weakening of their cell membranes. This, I have endeavored to show, is in its effect substantially identical with chilling.

6. THE TWIGS OF TREES AND SHRUBS AFTER THEIR WINTER CHILLING AND THE TRANSFORMATION OF THEIR STARCH INTO SUGAR MAY BE REGARDED AS MECHANISMS FOR THE DEVELOPMENT OF HIGH OSMOTIC PRESSURES WHICH START THE PLANT INTO GROWTH.

Food in the form of starch can not be utilized by a plant directly. The starch must be changed into sugar before it can be used in making new growth. But this transformation does more than make the starch available as food for the growing plant. It serves also to increase the tendency of the cells to swell and enlarge. In the form of starch the material is inert in the creation of osmotic pressures, but when transformed into sugar it becomes exceedingly active. According to the rigid experimental tests of H. N. Morse and his associates, a normal solution of cane sugar at 32° F. has an osmotic power of 25 atmospheres of pressure. It has been demonstrated that there sometimes occur in the cells of plants osmotic pressures as high as 30 atmospheres, or 450 pounds to the square inch, a pressure sufficient to blow the cylinder head off an

ordinary steam engine. It can hardly be questioned that these or even much lower osmotic pressures take an important part in forcing open the buds of once dormant plants.

We have evidence that there sometimes arise within the plant osmotic pressures of such intensity as to threaten the rupture of the cells. Consider the case of the exudation of drops of sugar solution from certain specialized glands. When this exudate of sugar occurs in flowers it is known as nectar, and it serves a useful purpose to the plant by attracting sugar-loving insects which unconsciously carry pollen from flower to flower and accomplish the beneficial act of cross-pollination. But sugar solution is often exuded outside the flower, in positions, or at times, that preclude any relation to cross-pollination. For example, a blueberry plant during its spring growth, when a leaf has reached nearly full size, is sometimes observed to exude drops of sugar solution from certain glands on the margins of the leaf and on the back of the midrib (Pl. 28). It is physically impossible for the sugar to have left the cells by osmosis. The sugar serves no useful purpose to the plant through the attraction of insects. The exudate certainly can not represent the elimination of a waste product, for sugar is one of the substances most used by plants in forming new tissues. I can conceive of no reason why the plant should exude sugar except to relieve a dangerous physiological condition—namely, the development of excessive osmotic pressures which would burst the cells of the plant or in some other way derange its physiological activities. I look upon such sugar glands as safety valves for the relief of excessive osmotic pressures that are dangerous to the internal economy of the plant. And not only is this conception applicable to extra-floral nectaries in general, but it may serve also, in the case of floral nectaries, to explain their origin. Having once arisen as osmotic safety valves, the usefulness of the floral nectaries as an aid to cross-pollination would then tend strongly to bring about their natural selection and perpetuation.

7. THE ESTABLISHMENT OF A DORMANT CONDITION BEFORE THE ADVENT OF FREEZING WEATHER AND THE CONTINUATION OF THIS DORMANCY THROUGH WARM PERIODS IN LATE FALL AND EARLY WINTER ARE PROTECTIVE ADAPTATIONS OF VITAL NECESSITY TO THE NATIVE TREES AND SHRUBS.

A little consideration will show how important the principle of chilling is to those species of trees and shrubs which are subjected each year to several months of freezing weather. If they were so constituted as to start into growth as easily in the warm days of late fall as they do in the warm days of early spring many species would come into flower and leaf in those warm autumn spells that we call Indian summer, and the stored food that the plant required for its normal vigorous growth in the following spring would be wasted in a burst of new autumn growth, which would be killed by the first heavy freezes and would be followed by a winter of weakness and probable death. But when two or three

months of chilling are necessary before a newly dormant plant will respond to the usual effect of warmth, such plants are protected against the dangers of growth in Indian summer. It is probable that all our native trees and shrubs are thus protected.

Any member of this audience may make a simple and instructive experiment next fall and winter with such early spring blooming plants as alder, hazelnut, pussy willow, yellow bush jasmine, forsythia, Japanese quince, peach, and plum. In mid-autumn bring into your living room and set in water freshly cut, dormant, leafless branches of these plants. They will not bloom. At intervals of a few weeks during late autumn and winter try the same experiment again. You will find that the branches cut at later dates will come into bloom under this treatment. They will not do so, however, until the expiration of the period of chilling appropriate to the various kinds of plants included in the experiment. The required period of chilling varies greatly. For some of the cultivated shrubs about Washington, especially the yellow bush jasmine (*Jasminum nudiflorum*), so brief a period of chilling is required that an extraordinarily cold period in late October or early November may chill them sufficiently to induce them to bloom if a period of warm weather follows in late November. The period of chilling required for the peach is so short that in Georgia unusually warm weather in December sometimes brings the trees into flower, and their crop of fruit is destroyed by the freezes that follow.

From these facts it appears that our native trees and shrubs are so intimately adjusted to the changes of the climate to which they have been long subjected that they are almost completely protected from injury by freezing, but some of the cultivated species brought from parts of the world having a climate different from ours are only imperfectly adapted to our climatic changes. They grow at times when our native species have learned to hold themselves dormant, and they often suffer severely in consequence.

Chilling, as a protective adaptation, has become a physiological necessity in the life history of cold-winter trees and shrubs. So fixed indeed, is the habit that it appears to be a critical factor in determining how far such plants may go in the extension of their geographic distribution toward the Tropics. In the Tropics our common northern fruit trees, apples, pears, peaches, cherries, grow well for a time and then become half dormant. In the absence of chilling they never fully recover from their dormancy; they grow with weakened vitality and finally die. If these fruits are to be grown successfully in the Tropics they must be given artificially the periodic chilling they require.

When it became evident from the earlier observations and experiments that chilling played so essential a part in the behavior of our trees and shrubs, it was clear that additional experiments ought to be conducted in which actively growing plants might be subjected to chilling

temperatures without being put in a dark place like the ordinary refrigerator. To meet the requirement of both cold and light a glass-covered, outdoor, brick chamber was constructed in 1912. It was kept above freezing by heating with electric lights, which were turned on and off automatically by a simple thermostat. In summer the chamber was kept cool, though not really cold, by means of ice and electric fans. Although much was learned with this apparatus, it was crude and inadequate. To provide for more exact experiments a glass-covered compartment chilled by a refrigerating machine was constructed in one of the Department of Agriculture greenhouses. The refrigerating apparatus is a sulphur-dioxid machine having a refrigerating power equivalent to 1,000 pounds of ice a day. It is run by a 2-horsepower electric motor, and it furnishes ample refrigeration for the lighted compartment, which is a glass-covered frame 25 feet long, 3 feet wide, and 14 to 20 inches in depth. The first of these refrigerated frames was devised and constructed in 1916. In this enterprise I had the valued advice and assistance of Dr. Lyman J. Briggs. The usefulness of this refrigerated frame in experimental work with plants was so great that another similar equipment was installed in 1918.

With the aid of this apparatus many of the experiments described in this address have been carried on or verified, as well as other experiments of a related character. For example, at ordinary summer temperatures many kinds of seed will not germinate but remain dormant until death overtakes them. Under the influence of chilling, however, these seeds are stimulated to prompt germination. (See Pl. 29.)

The experiments thus far made indicate the importance of a much wider use of the principle of chilling in many lines of experimentation bearing on the improvement of horticultural and agricultural practices. I commend the subject of chilling to experimenters in these lines, and I wish to call especial attention to the desirability of determining proper temperatures for the storage of seeds, bulbs, cuttings, and grafting wood, proper temperatures for the treatment of plants which are to be forced from dormancy to growth at unusual seasons, and proper temperature for the storage of nursery stock so that the nurseryman may have plants in proper condition for shipment on any date he desires. (See Pl. 30; 31, B; 32.)

The whole question of the effect of chilling on herbaceous perennials is an open field.

An understanding of the process of chilling explains the reason of some of the practices of gardeners, which they as well as botanists have erroneously ascribed to the need of "resting." What a gardener calls "resting" is often in reality a period of chilling, characterized not by physiological rest but by pronounced internal activity. Rest alone would not, in the case of our cold-climate trees and shrubs, accomplish the purpose the gardener has in mind. It is chilling, not rest merely, that

is required. The practice of gardeners and nurserymen known as the "stratification" of seeds is probably to be explained as in reality a process of chilling.

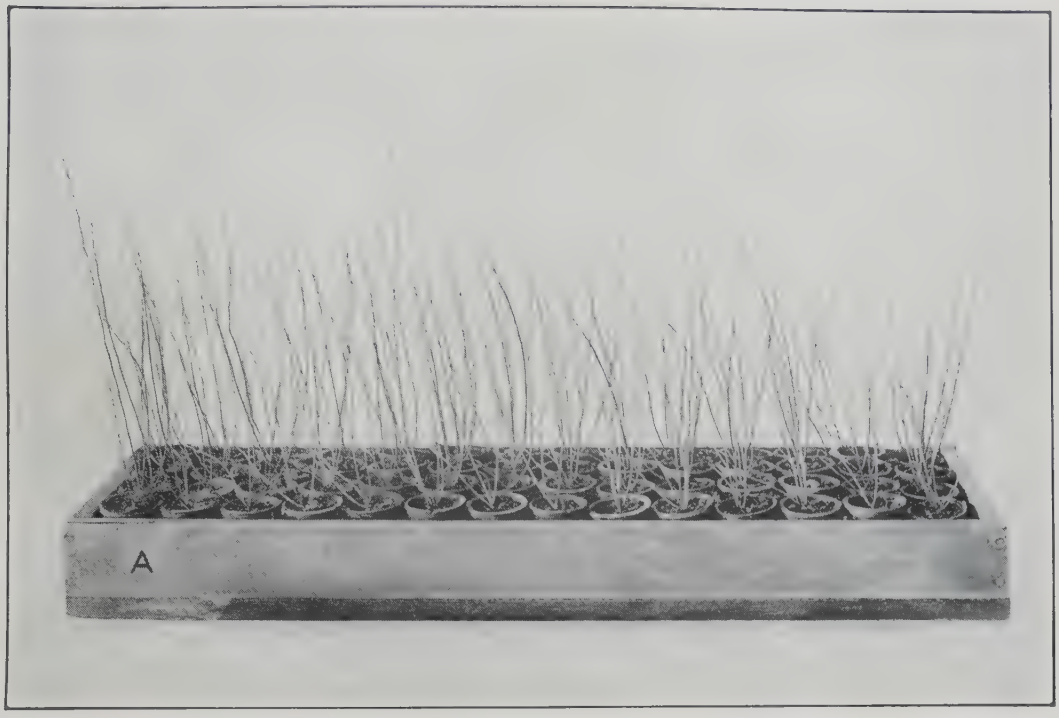
As a single example of the application of the principle of chilling let me cite the case of the blueberry. For several years we have been trying at the Department of Agriculture to domesticate this wild plant. We have raised many thousand hybrids and have set them out in waste sandy lands in the pine barrens of New Jersey (Pl. 33, A). We have grown the bushes to fruiting age and have brought them into highly productive bearing (Pl. 33, B). We have made them fruit so lusciously and so abundantly that they have brought returns to the grower at the rate of more than $1,000 an acre. In a word, we have changed the blueberry from a small wild fruit the size of a pea to a fruit the size of a Concord grape, and we have made its culture a profitable industry. (See Pl. 34, 35.) These things we should not have been able to do unless we had first worked out the principle of chilling, an understanding of which was essential to our work of breeding and propagation.

In conclusion, I wish to express the opinion that the chilling of dormant trees and shrubs of temperate climates as a prerequisite to their resumption of normal growth in the spring ought to be recognized in books on plant physiology as one of the normal processes in plant life. These works should contain chapters on chilling, just as they now contain chapters on other fundamental factors and principles relating to the life history of plants. And especially in books on plant physiology in relation to agriculture should the subject of chilling be dealt with in detail, for when in the pursuit of agriculture we take plants from one part of the world to another, or undertake to grow them out of season, or attempt to propagate them in quantity by grafting or by other processes unknown in nature, we are greatly handicapped and limited in our operations if we do not understand the principles of a process so widely existent in nature and so indispensable to a large proportion of the plants of temperate agriculture as the process of chilling.

PLATE 20

A.—Blueberry plants, *Vaccinium corymbosum*, made dormant without cold. These blueberry seedlings, in 2-inch pots, were kept during the fall and winter in a greenhouse at a temperature of 55° to 70° F. Although this is a very favorable temperature for the growth of the blueberry, these plants shed their leaves and became completely dormant, just as they ordinarily do when exposed to the frost and cold of an outdoor fall and winter. The photograph was taken on January 25.

B.—Chilled and unchilled blueberry plants. The six blueberry plants at the left, after an outdoor winter chilling, were brought indoors on March 25, into a greenhouse having a temperature of 55° to 70° F., and were repotted. On April 20, when the photograph was taken, they had developed both leaves and flowers, while the six plants at the right, which had been in the same greenhouse at the same temperature all the fall and winter and were repotted on the same date as the others, were still completely dormant.


A


B


A


B

PLATE 21

A.—Chilled and unchilled plants of grouseberry, *Viburnum americanum*. The illustration shows two 1-year-old seedlings with the same history, except that the one at the right was kept during the winter in a warm greenhouse at a temperature of 55° to 70° F., while the one at the left was wintered in a cold greenhouse at a temperature of 32° to 40°. When spring temperatures warmed up this coldhouse, the plants in it began to grow, and on April 7, 1914, when the photograph was taken, they had reached the stage shown in the left-hand figure, while the plants in the warmhouse, as illustrated by the right-hand figure, were still completely dormant.

B.—Chilled and unchilled plants of tamarack, *Larix laricina*. These two seedlings, grown from seed procured in Alaska, have had the same history except that the one at the left was wintered in a cold greenhouse at a temperature of 32° to 40° F., the one at the right in a warm greenhouse at a temperature of 55° to 70°. When the photograph was taken, on April 10, 1914, the chilled plant had put out new growth in the warm spring weather, while the unchilled plant still showed only its leaves of the year before.

PLATE 22

A.—Chilled and unchilled plants of wild crab, *Malus coronaria*. The plant at the left had been outdoors during the fall and winter, leafless and dormant, exposed to the frost and cold. The plant at the right had been in the warm greenhouse during the fall and winter at a temperature of 55° to 70° F. When the outdoor, chilled plant was brought into the greenhouse in early spring, it promptly began to put out new leaves and twigs, but the indoor, unchilled plant continued its dormancy. The photograph was taken April 24, 1917.

B.—Blueberry plant with one branch stimulated to growth by cold. The right-hand branch has been stimulated to growth by chilling; the left-hand branch has been kept dormant by heat. For a detailed description of this experiment see p. 152–153.


A
B


B

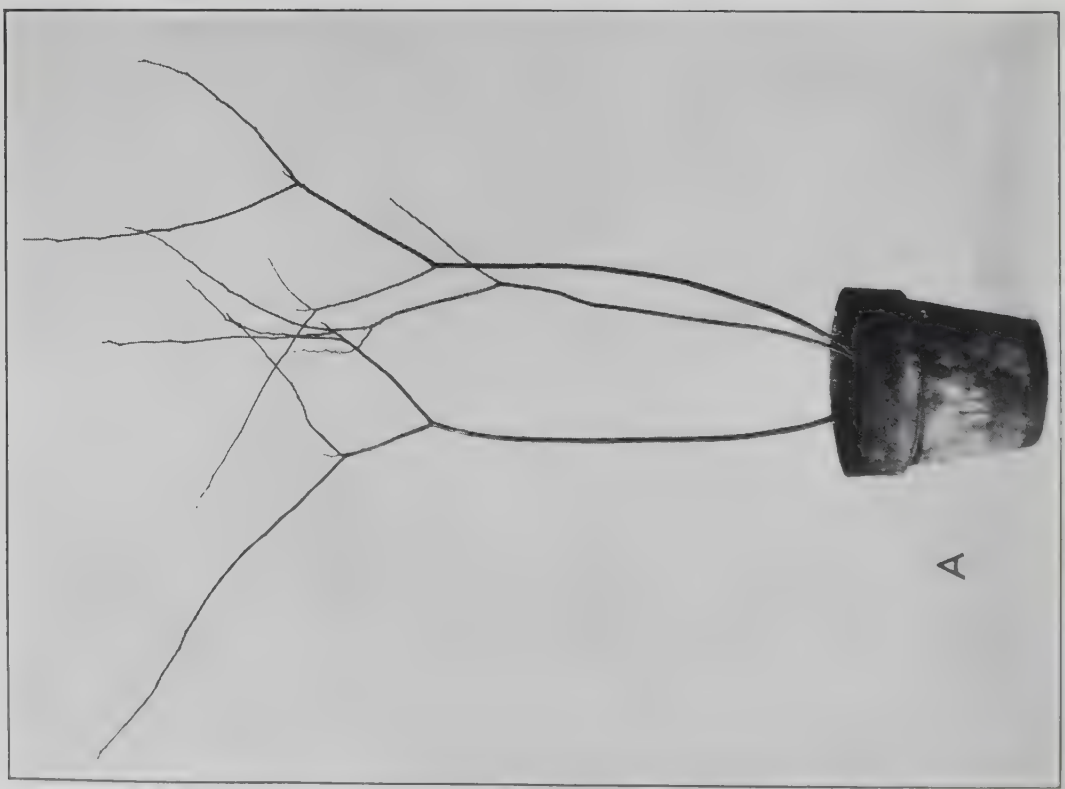

A

PLATE 23

Blueberry plant with one branch kept dormant by heat.

A.—Dormant indoor blueberry plant as it appeared on February 15, 1912. On that date the pot containing the plant was placed on a shelf outside a greenhouse, and a single branch was passed through the glass wall into the warm interior.

B.—Same plant photographed May 21. When spring came, all the outside branches, which had been chilled, burst into normal leaf, while the branch inside the greenhouse, which had been kept warm, still remained dormant.

PLATE 24

A.—Blueberry cuttings starting to grow at 36° F. These cuttings were placed in cold storage while still completely dormant. Although the temperature did not go above 36° F., buds on each of the cuttings finally began to grow. It is to be noted that although growth took place in the buds the other kind of growth which results in the formation of a callus, or healing-over tissue, at the severed base of the cutting is wholly lacking. Callusing can not take place at so low a temperature.

B.—Blueberry plant growing in the dark at 36° F. This plant was in cold storage in the dark in a commercial refrigerating establishment from March 30 to December 4, 1915. The temperature ranged from 33° to 36° F. Some of the plants in this experiment made new growth to the length of 32 mm.


A


B

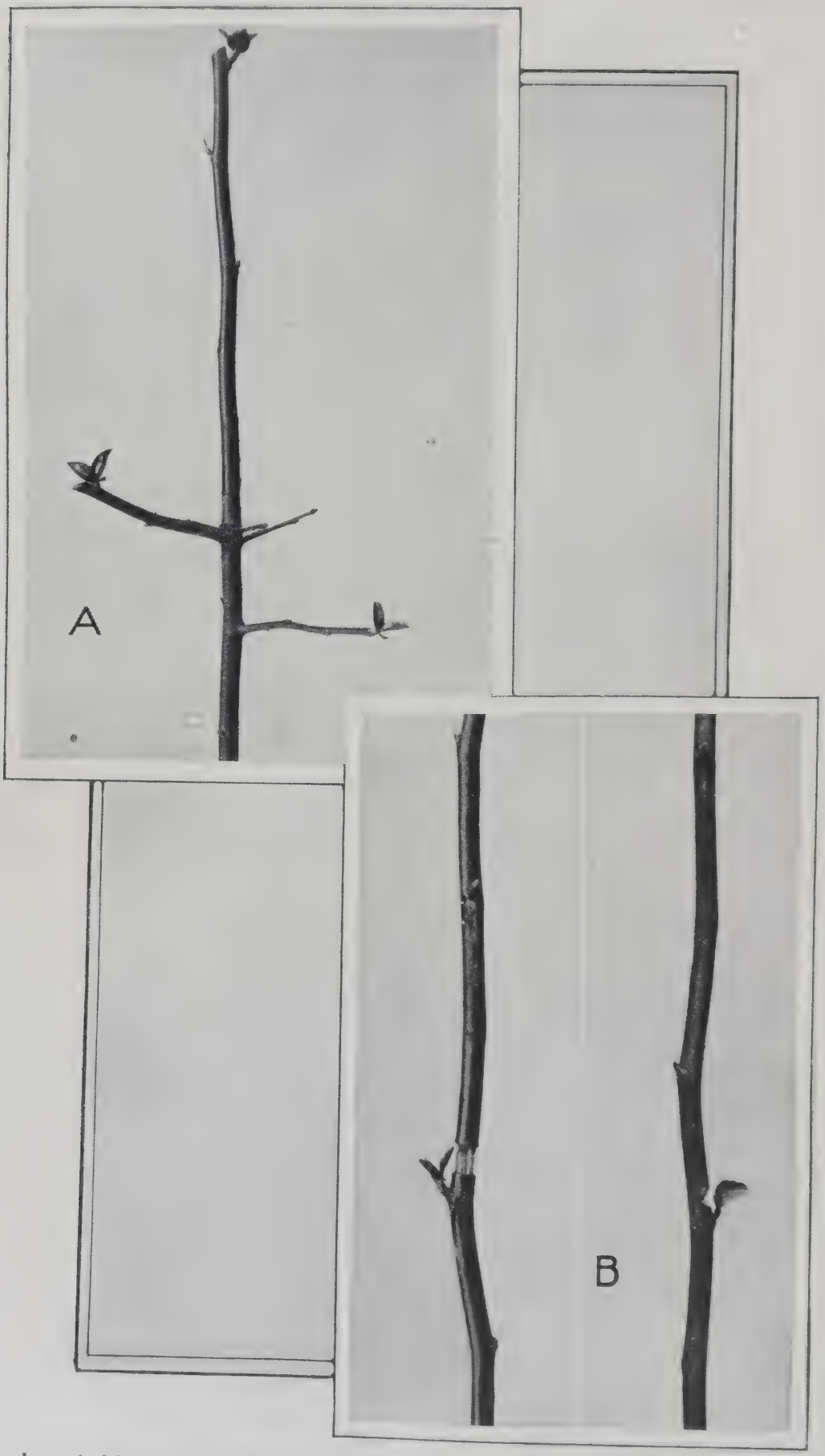

A
B

PLATE 25

A.—Dormant wild crab stimulated to growth by pruning. This plant had remained dormant in the warm greenhouse during the fall and winter at a temperature of 55° to 70° F. On April 5 three branches were pruned, and on April 24, when the photograph was taken, the uppermost bud on each of the pruned branches had begun to grow. On other, unpruned plants no bud growth had taken place.

B.—Dormant wild crabs stimulated to growth by girdling and by notching the stem. These plants had had the same preliminary treatment as the one illustrated in A—that is, they had been kept in the warm greenhouse all winter, without chilling. On April 4 a ring of bark was removed from the plant shown in the left-hand figure, and the soft cambium was carefully scraped away, down to the hard wood. On April 24, when the photograph was made, the bud next below the girdle had begun to push. The stem of the right-hand plant was notched in early April. The bud next below the notch soon began to grow. The photograph was taken on May 2.

PLATE 26

A.—Dormant blueberry buds stimulated to growth by chalking the stem. This plant was brought into the greenhouse February 4, 1913, to be used in breeding experiments. It flowered, but since it had been insufficiently chilled only a few of the uppermost leaf buds on each stem grew. In order to keep small ants from crawling up the stems and interfering with the pollination experiments the stems were chalked near the middle. The dormant buds in and just below the chalked areas started growing. The photograph was taken April 5, the stems being rechalked over the same areas that were originally chalked. After numerous repetitions of the experiment it was found that if the chalking was done lightly the buds would not grow, but if the stems were rubbed hard in the process of chalking, as commonly happened in the case of very smooth stems, the buds grew. It was the hard rubbing, not the chalk, that stimulated the growth.

B.—Dormant blueberry bud stimulated to growth by rubbing the stem. The photograph, which was taken June 14, 1913, shows a single bud starting into growth on a dormant blueberry plant. The dark area just above the bud is a brown band on an otherwise green stem. It shows the position of a rubbing that was given the stem with a smooth knife handle a few weeks earlier. This bud afterwards grew into a long, vigorous branch, while all the other buds remained dormant.


A
B


A
B

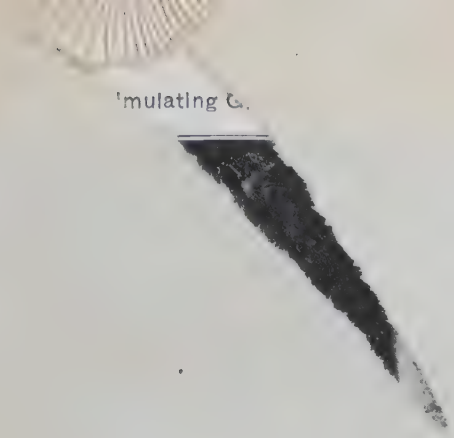


PLATE 27

A.—Normal spring growth on a blueberry stem. This illustration is from a photograph taken April 24, 1909. In the preceding season the plant had sent up an unbranched shoot. After an outdoor chilling through the winter and early spring it put out flowers and new twigs as shown in the illustration. The fact to be especially noted is that the new growth on this stem took place from numerous buds.

B.—Abnormal spring growth on a blueberry stem, due to lack of chilling. This photograph was taken on May 19, 1913. Growth is taking place from only one bud, the third from the tip. The uppermost bud is a flowering bud, the second a leaf bud. Both are dead or dying. This plant had stood in the warm greenhouse all winter and spring. If it had had the usual two to three months' chilling its starch would have been transformed into sugar and the stem would have flowered and put out new twig growth from numerous buds in the same manner as the stem shown in A.

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PLATE 28

Blueberry leaf exuding sugar from glands interpreted as osmotic-pressure safety valves.

This is a leaf of the highbush blueberry, *Vaccinium corymbosum*. The photograph was taken May 19, 1916. The sugar-secreting glands, sometimes called extra-floral nectaries, are situated in this plant on the back of the midrib and along the margins of the leaf, toward its base. The drops of sugar solution have been wiped away from the glands on the left-hand margin and from two glands on the midrib at the base of the second and fourth lateral veins above the sugar drop shown near the middle of the picture. × 4.





PLATE 29

A plant of bunchberry, *Cornus canadensis*, the seeds of which do not germinate without chilling.

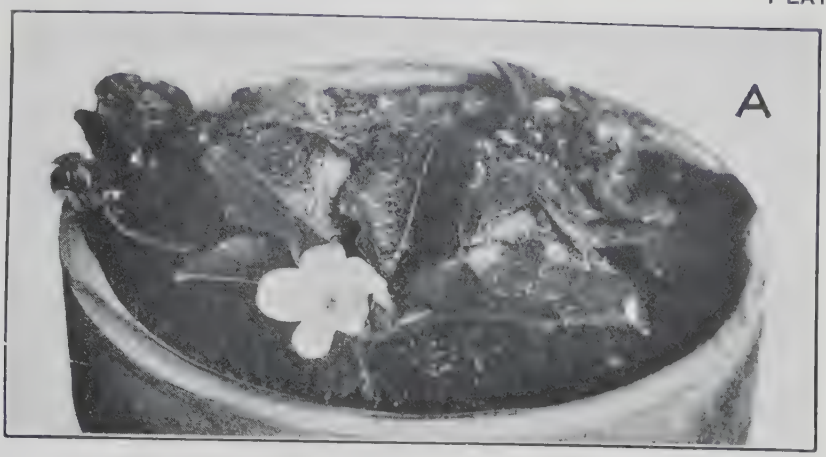
Bunchberry seeds sown October 9, 1912, and chilled during the winter germinated promptly the following spring. Another lot of the same seeds sown on the same date but kept in a greenhouse at a temperature of not less than 55° F. showed no germination in 12 months. These seeds were then chilled for 2 months at a temperature of 35° to 40° F., and when brought back into the greenhouse they germinated within a month. The very healthy plant shown in the illustration grew from one of these long-dormant seeds. The exposure of seeds to winter weather is sometimes practiced by gardeners, but they usually attribute its beneficial effect to freezing, which in all the cases tried in these experiments is unnecessary.

PLATE 30

A.—Trailing arbutus, *Epigaea repens*, flowering sparingly from lack of chilling. This plant of trailing arbutus was grown from seed. In the autumn, when about a year old, it laid down clusters of flowering buds. It was kept in a warm greenhouse all winter, but when flowering time came most of its flower buds were dead and brown. Only a single flower opened.

B.—Trailing arbutus plant flowering normally after chilling. This plant had the same history as the plant described under A, except that it was kept outdoors during the winter and brought back into the greenhouse in the spring. At the age of 14 months, when the photograph was taken, March 27, 1911, the plant was in full flower, healthy and normal.

C—Blueberry plant forced into flower in September by artificial chilling. This plant was brought indoors in late winter. It made new growth, and during the cool weather of May it laid down flowering buds for the next year, as a blueberry plant ordinarily does in autumn. During the summer, however, the plant was given an artificial winter by chilling it for three months in an artificially refrigerated glass-covered frame exposed to daylight. When brought out of the frame, in September, the plant promptly flowered, as shown in the illustration.


A


B


C

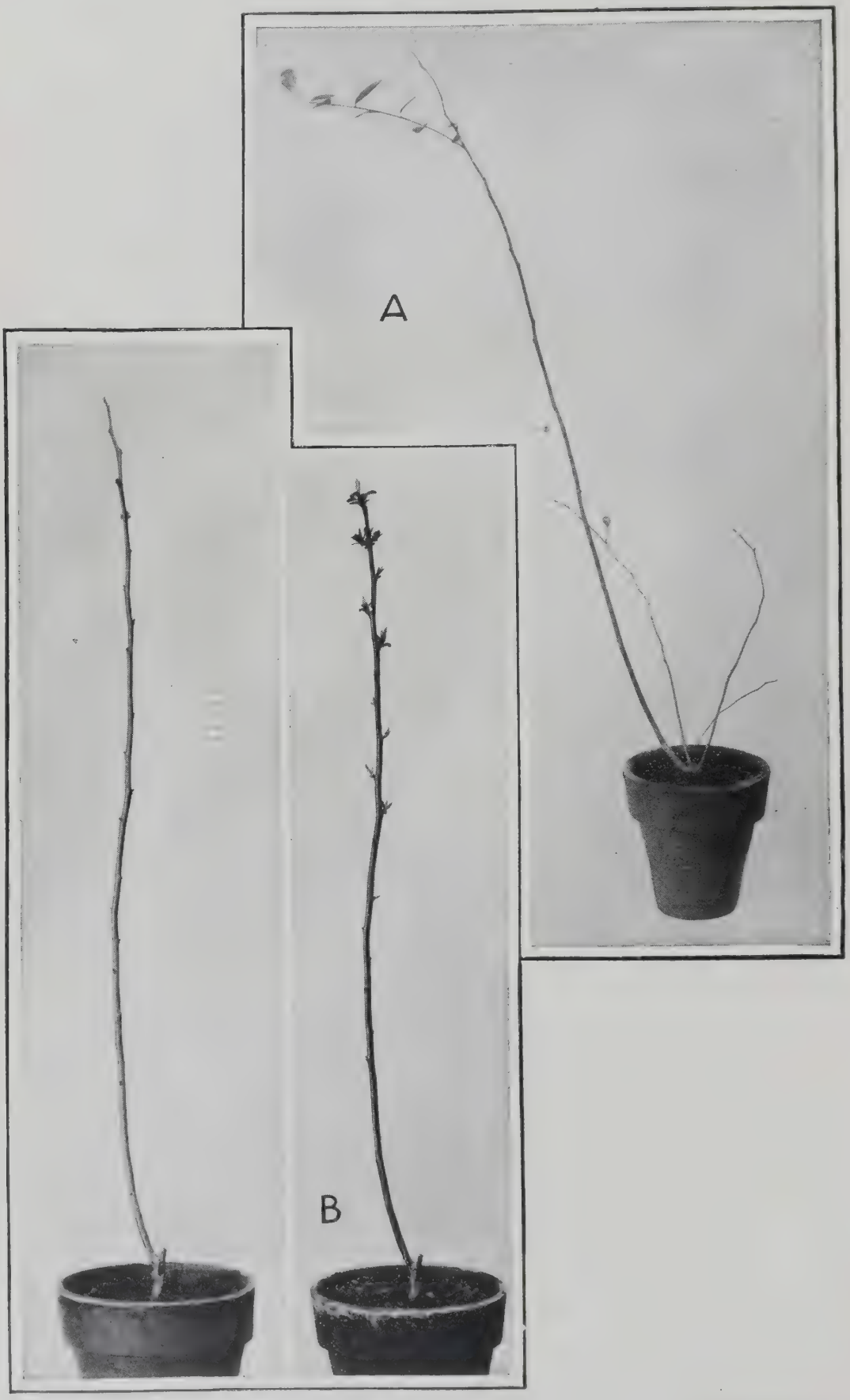

A
B

PLATE 31

A.—Abnormal growth of an unchilled blueberry plant. This plant became dormant in the autumn in a warm greenhouse, and since it was not chilled it continued its dormancy through spring and summer for a period of nine months. Then three of its stems began to die at the tips and, following this, growth began to take place from a single bud next below the dying tip on each stem. For the explanation of this abnormal activity see p. 156. The photograph was taken October 12, 1916.

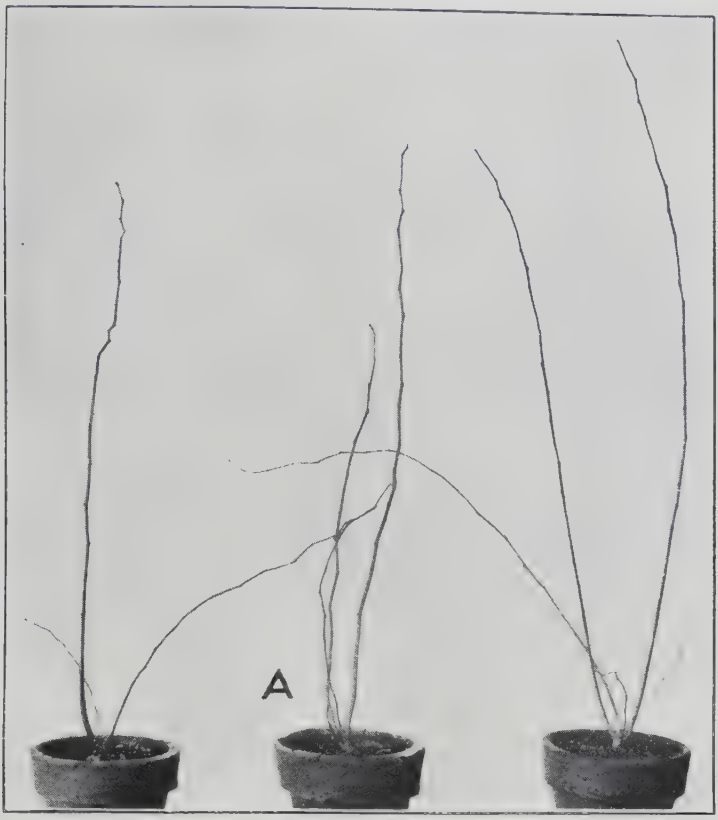
B.—Awakening of long dormant plants by artificial chilling. The illustration consists of two photographs of the same plant. At the left is shown the condition of the plant on December 26, 1916, after more than a year of warmth and dormancy. The figure at the right, from a photograph taken April 27, 1917, shows the appearance of the plant after it had been subjected to artificial chilling for a period of three months and then had been returned to the warm greenhouse. It began to put out new growth from 10 or more of its leaf buds. Even after its extraordinarily long period of dormancy the plant had been brought back to normal activity by a suitable period of chilling.

PLATE 32

Plants brought out of dormancy at a specified time.

A.—Blueberry plants from a lot that had been kept in a dormant condition by warmth for nearly a year. On October 30, 1917, plants from this lot were placed under chilling conditions at a temperature of about 35° F. At the end of a month's chilling eight plants were taken out, repotted, and brought into a greenhouse maintained at a temperature of 50° to 70° F., and after two months' chilling eight other plants were brought out.

B.—Representative plants from each of the two chilled lots described under A, from photograph made January 18, 1918. The plant at the left, which was kept under refrigeration for a month, was only imperfectly chilled, and although it started growing the growth was from abnormally few buds. But the plant at the right, under refrigeration for two months, was adequately chilled and started into growth from many buds in a normal manner. It is evident that by the proper application of this procedure a plant of this nature can be brought into proper condition for shipment and planting on any date desired.


A


B


A


B

PLATE 33

A.—Plantation at Whitesbog, N. J., for the testing of blueberry hybrids. From very carefully selected wild blueberry plants hybrid seedlings are raised in the greenhouses of the Department of Agriculture at Washington. In order to bring them into fruit under favorable outdoor conditions so that selections of the best hybrids can be made for further propagation, the young seedlings are sent to a plantation at Whitesbog, 4 miles east of Browns Mills, in the pine barrens of New Jersey. In the photograph 2-year-old hybrids are shown at the right and 3-year-olds in the row at the left.

B.—Four-year-old blueberry hybrid in full fruit. This illustration shows the vigor, beauty, and productiveness of a hybrid blueberry bush when it is given the proper and peculiar conditions which by its nature it requires for successful growth. From a ⅓-acre patch of hybrid bushes a yield of berries was secured in 1919 at the rate of 96 bushels per acre. They sold at a little over $10 a bushel, bringing gross receipts at the rate of $966 per acre. In 1920 this planting yielded at the rate of 117 bushels per acre, which sold at a little less than $11 a bushel, yielding gross receipts at the rate of $1,280 per acre.

PLATE 34

The ordinary wild blueberry of New Jersey.

This is a photograph, natural size, of a quart box of wild New Jersey blueberr rather better than the average. It was taken for the purpose of comparison with selected hybrid blueberries shown in Plate 35.



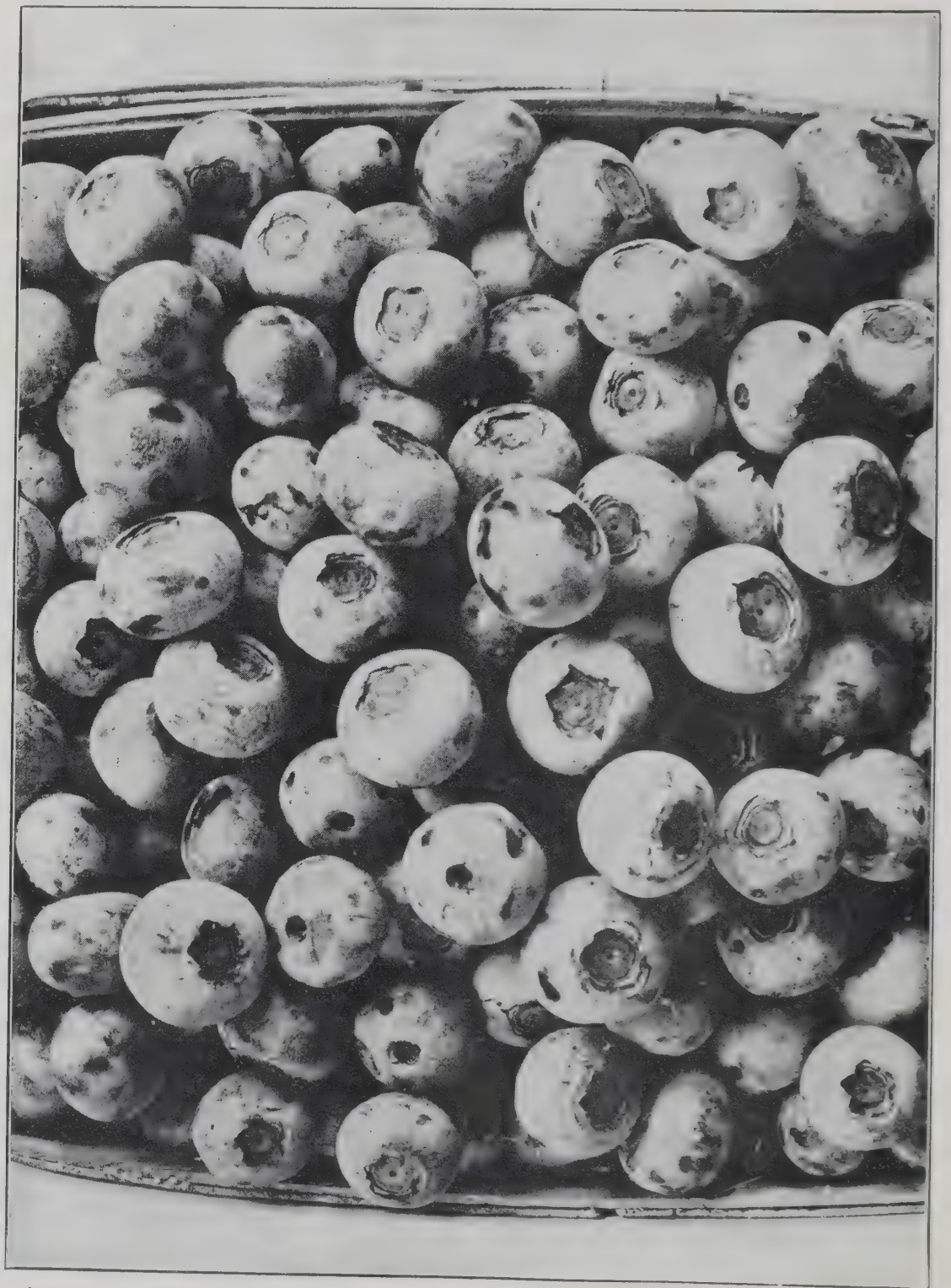


PLATE 35

Fruit of a selected hybrid blueberry.

This illustration shows in natural size a quart box of blueberries from a hybrid produced at Washington and fruited at Whitesbog. The photograph represents the average product of the bush, for it was taken from a clean picking, including the small berries as well as the large ones. Hybrids with berries of still larger size have been fruited at Whitesbog.

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COMPOSITION OF NORMAL AND MOTTLED CITRUS LEAVES[1]

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INTRODUCTION

Knowledge concerning the composition of a plant is essential to an understanding of its growth. The amounts and proportions of the different constituents absorbed from the soil or other nutrient medium, as revealed by accurate analysis of the several parts of plants, undoubtedly give some indication concerning their nutritional requirements. If determined progressively, such data may contribute to a clearer understanding of fundamental physiological processes of growth.

The interpretation of plant analyses, so far as growth processes and requirements are concerned, demands great caution, however. Many plants undoubtedly have the power of adapting themselves to a wide range of soil variations; and the composition of the plant, owing to selective absorption, commonly bears little direct relation to the composition of the nutrient solution. It is well known that the concentration of a given constituent in the nutrient solution may be varied considerably without producing any material change in the composition of the plant.

The effect of an excess or deficiency of one ion on the absorption of other ions, and especially the effects of nonessential salts on the absorption of essential ions, have not been sufficiently studied. Despite the many investigations during recent years on antagonism, comparatively few analyses have been made showing the effects on absorption. Likewise, investigations on the so-called nutritional or physiological diseases have not dealt with absorption specifically, except to a very limited extent.

Previous studies on the rate of absorption of nutrients have been conducted mainly with annual plants, chiefly cereals, very limited study having been devoted to trees. There is much need for accurate data on the several phases of absorption as related to the growth of fruit trees.

[1] Paper No. 67, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

In connection with investigations on the nutrition of different species of citrus trees, especially as related to that condition known as mottle-leaf, we have determined the composition of different parts of the tree, such, for example, as the roots, old wood, young wood, leaves, leaf sap, and fruit. This work has extended over a period of several years, and further study is contemplated. Some of the results already obtained have proved to be of special interest. The present paper will be devoted mainly to a discussion of the composition of the leaves.

It is not necessary to review the many published analyses of citrus fruits. Most of the publications on this subject have dealt mainly with the organic constituents and total ash, with an occasional analysis of the ash. Comparatively few analyses have been published showing the composition of portions of citrus trees other than the fruit.

The earliest investigation we have been able to find, and perhaps the best known, is that of Rowney and How (*15*)[1], published in 1848. Analyses were reported of the roots, stems, leaves, and fruit of orange trees, *Citrus aurantium*, grown on the island of St. Michael. The variety was presumably that now known as St. Michael.[2] The analyses were expressed as percentages of the carbon-dioxid-free ash. The results were similar to our analyses of California orange trees, when calculated to the same basis.

In 1891 Oliveri and Guerrieri (*13*) published an extended study on the composition of the wood, leaves, and different portions of the fruit of the orange, *Citrus aurantium* Riss;[2] Mandarin, *C. nobilis* var. *deliciosa*, Swingle; and lemon, *C. limonia* Osbeck, grown in Palermo, Italy. This investigation, extending over a period of three years, is the most complete study yet published on the composition of different parts of citrus trees. They recorded the number and weights of fruits produced by different classes of trees and the number and weights of leaves and the weights of wood pruned from the trees during a period of three years, representative samples of which were analyzed. Some of their analyses also agree reasonably closely with our data.

In 1901 Aliño (*1*) determined the phosphoric acid, potash, and nitrogen content of orange wood, leaves, and fruit; and in 1909 Muller (*12*) published complete analyses of seedling orange leaves from healthy and diseased trees grown in South Africa.

In 1910 Blair (*2*) analyzed orange leaves and stems grown in Florida. His samples represented the new growth taken in October from certain plots of a fertilizer experiment. In 1917 Jensen (*7*) published a paper on the composition of normal and mottled orange, lemon, and grape-

[1] Reference is made by number (italic) to "Literature cited," p. 190–191.

[2] In this case, the sweet orange, *Citrus sinensis* Osbeck, is doubtless the species studied. W. T. Swingle's revision of citrus nomenclature, as given in the "American Standard Cyclopedia of Horticulture," is followed in this paper.

fruit (*Citrus grandis* Osbeck) leaves grown in California. Further reference will be made to this paper later.

As is well known, the composition of annual herbaceous plants depends on their age. It has been shown that the ash content and the proportions of the individual constituents absorbed from the soil change as growth proceeds. Of the changes in perennials much less is known. It seems reasonable to suppose, however, that the growth processes are similar. The periodically developing new shoots may be likened to the portion of annual plants growing above ground.

New shoots appear on citrus trees several times each year. The tree, being evergreen, bears leaves at all seasons. Consequently, the foliage is composed of leaves of different ages. A given leaf ordinarily remains on the tree for a period of from two to three or more years.

SELECTION OF SAMPLES

Special care has been taken to secure representative samples of leaves of known age. Familiarity with the appearance of developing citrus leaves proved to be a material aid in selecting the samples. A considerable portion of the samples were obtained from trees growing near the laboratory where daily observations were made. The leaves of the Washington Navel and Valencia orange, the Eureka lemon, and the Marsh seedless grapefruit have been analyzed. Each sample was composed of several hundred leaves, collected from six or more adjacent trees, all of which were reasonably uniform in appearance and the culture and fertilization of which had been the same. The trees were 10 or more years of age. The entire leaf, including the petiole, was analyzed as a unit.

The samples were picked from the trees, placed in tight bags and immediately taken to the laboratory and weighed. In most cases this procedure did not require more than 30 minutes. In order to remove dust and other adhering foreign material, the leaves were thoroughly cleaned by wiping each leaf with a moist cloth, but washing with water was necessary with a few samples heavily coated with dust or showing evidences of residues from previous spraying. Early in this work it was found that the samples from which the dust had not been completely removed contained abnormally high percentages of silica, alumina, iron, and inorganic materials not soluble in dilute hydrochloric acid.

METHODS OF ANALYSIS

The samples were dried at 105° C. for 24 hours, and the loss in weight was calculated as moisture. The dry samples were ground to a powder in a small hand mill, were thoroughly mixed, and were then stored in tightly stoppered bottles for analysis.

Total nitrogen was determined by the official Kjeldahl method, modified to include nitrates. Total sulphur was determined by the sodium-peroxid

fusion method. The fusions were made over alcohol flames, and the sulphate was precipitated as barium sulphate, usually from the solution of the entire mass used in making the fusion. Total phosphorus was determined by treating 1 to 2 gm. of the dry material with a solution of magnesium nitrate, evaporating to dryness, igniting, and proceeding in the usual manner. Chlorin was determined in a special portion of the ash made by igniting at a low heat 5 to 10 gm. of the dry material, dissolving the residue in dilute nitric acid, and proceeding with the Volhard volumetric method. In some cases chlorin was also determined by performing the incineration in the presence of an excess of sodium carbonate in order to avoid the possible loss of chlorin, but the results of the two methods were similar.

For the determination of total ash, 10 to 20 gm. of the dry samples were incinerated in porcelain dishes over Bunsen burners. The material charred easily and burned quietly upon the application of low heat and was reduced to a gray ash without approaching dull redness. The residue was then allowed to cool, was taken up with hot water, transferred to a filter, and washed thoroughly. The insoluble material with its filter paper was transferred to a platinum dish, dried, pulverized with an agate pestle, and heated to full redness. When the platinum dish cooled, the filtrate from the previous leaching was added and evaporated to dryness. Ten to 20 cc. of strong ammonium-carbonate solution were then added, and the treatment was repeated until the ash was completely carbonated, as was indicated by constant weight upon evaporating to dryness and heating gently. The results are recorded as percentages of ash. It should be stated that the ash thus obtained differs from that reported by other investigators in that we are dealing with completely carbonated ash, whereas previous analyses of citrus leaf ash have been calculated to a carbon-dioxid-free basis.

The ash was dissolved in water and dilute hydrochloric acid, and the solution was evaporated to complete dryness on the water bath in order to dehydrate the silica. The amount of uncombined carbon found in the ash was always entirely negligible. The residue was taken up with warm water and dilute hydrochloric acid. The silica was determined by the loss in weight occasioned by treating the incinerated residue with hydrofluoric acid. The material nonvolatile in hydrofluoric acid usually amounted to only 0.1 to 0.2 per cent of the ash and was neglected in this work. The filtrate from the silica separation was made up to a definite volume, usually 500 cc., and the various constituents were determined in aliquots representing from 0.2 gm. to 0.4 gm. of the ash.

The methods of the Association of Official Agricultural Chemists [1] were used with slight modifications, as noted. Iron, aluminum, and

[1] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig., 1908. Reprinted in 1912.

phosphoric acid were precipitated collectively by adding a weighed excess of ferric chlorid, neutralizing with ammonia, filtering, redissolving in dilute hydrochloric acid, and repeating the process. Iron was precipitated with ammonia from a separate aliquot and determined volumetrically by reduction with zinc and titration with permanganate. This method was occasionally supplemented by the ferrocyanid colorimetric method with fairly satisfactory results. Aluminum was calculated by difference after the phosphoric acid was gravimetrically determined in a separate aliquot. Calcium, magnesium, potassium, and sodium were determined in the filtrate after the ammonia precipitate was removed, and in some cases manganese was determined by bromin oxidation. Sulphate was determined gravimetrically in an aliquot of the original solution. Carbon dioxid was not determined.

COMPOSITION OF NORMAL MATURE ORANGE LEAVES

A considerable number of analyses have been made of mature orange leaves representing both the Washington Navel and Valencia varieties. Owing to the absence of previous records showing the age of the leaves available for analysis, and in view of the fact that orange leaves, when from 4 to 6 months of age, assume an appearance not unlike that of leaves 1, 2, or more years of age, it is highly probable that random samples will always represent mixed ages.[1] Most of our samples of mature leaves were taken at random, always avoiding immature or abnormal individuals. The samples were gathered at different seasons of the year and from a considerable number of different sets of trees, some of which were growing in different localities. Typical analyses are submitted in Tables I and II.

It is interesting to note that the composition of the different samples was found to be reasonably uniform despite the fact that their average ages, although they were mature in appearance, probably varied considerably. Other samples not reported above showed a similar composition. The data also afford but little evidence of seasonal variation in composition.

Except in calcium and potassium content, the different samples of the same variety differed almost as widely in composition as the samples of different varieties. The samples from different localities were also similar in composition, although those from Riverside were grown on sandy loam soil, that from Anaheim on light sandy soil, and the one from Whittier on heavy adobe.

It will be noted that the average calcium content of Valencia leaves was found to be somewhat higher than that of Navels, while the reverse is true for potassium.

[1] Ensign (*6*) has recently shown that the size of the vein islets of *Citrus grandis* is directly correlated with the maturity of the leaf. From the most immature to fully matured leaves there is a gradual increase in the size of the vein islets. If further investigation prove that similar relations occur in other species of citrus, a direct means will be afforded by which the age of the leaves can be determined.

TABLE I.—*Composition of mature normal orange leaves*

NAVEL

Locality.	Date collected.	Probable age (in months).	Constituents of ash.								
			SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Riverside, plot U	Dec. 12, 1916	6 to 24...	2.17	0.14	30.68	1.53	7.33	0.83	2.83	2.85	0.24
Riverside, plot V	do	do	2.54	.18	31.67	1.84	5.13	1.07	2.80	3.65	.39
Riverside, plot U	May 21, 1917	6 to 12...	1.54	.14	31.89	1.81	6.67	.43	2.27	2.25	1.13
Average			2.08	.15	31.41	1.73	6.38	.78	2.63	2.92	.95
VALENCIA											
Arlington	Jan. 29, 1917	6 to 24...	2.94	0.18	33.79	2.82	4.11	0.90	2.46	2.52	0.24
Riverside, plot U	May 23, 1917	6 to 12...	2.25	.10	34.40	1.75	3.33	.48	1.97	2.47	.56
Whittier	Feb. 1, 1917	6 to 24...	1.17	.07	35.58	1.64	3.68	.40	2.16	2.64	.15
Anaheim	Aug. 29, 1917	12 to 24..	3.22	.11	32.97	1.32	5.45	.62	1.76	2.46	.19
Average			2.39	.11	34.19	1.88	4.14	.60	2.09	2.52	.28

TABLE II.—*Composition of mature normal orange leaves*

NAVEL

Locality.	Date collected.	Probable age (in months).	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
					SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
			Per cent.	*Per cent.*										
Riverside, plot U	Dec. 12, 1916	6 to 24...	59.58	15.39	0.34	0.021	4.73	0.24	1.13	0.13	0.14	0.45	2.70	0.04
Riverside, plot V	do	do	58.86	15.23	.39	.028	4.82	.28	.78	.16	.13	.42	2.58	.06
Riverside, plot U	May 21, 1917	6 to 12...	62.35	16.33	.25	.022	5.21	.29	1.09	.07	.14	.25	2.12	.18
Average			60.26	15.65	.33	.024	4.92	.27	1.00	.12	.14	.37	2.47	.09
VALENCIA														
Arlington	Jan. 29, 1917	6 to 24...	55.40	14.38	0.42	0.026	4.86	0.21	0.59	0.13	0.11	0.30	2.31	0.03
Riverside, plot U	May 23, 1917	6 to 12...	61.49	16.45	.37	.017	5.66	.29	.55	.08	.17	.27	2.02	.09
Whittier	Feb. 1, 1917	6 to 24...	58.77	18.25	.21	.013	6.49	.30	.67	.07	.20	.36	2.41	.03
Anaheim	Aug. 29, 1917	12 to 24..	63.49	18.90	.61	.021	6.23	.25	1.03	.12	.18	.34	2.23	.03
Average			59.79	16.99	.40	.019	5.81	.26	.71	.10	.16	.32	2.24	.04

Throughout this work we have determined the aluminum. Qualitative tests usually indicated this element to be present, but the quantity was never more than a few tenths of 1 per cent of the ash. Frequently the amount was undeterminable. The manganese was also determined in several samples. The amount was found to vary from 0.1 per cent to 0.2 per cent of the ash.

The size of the leaves as gauged by their average weights was recorded, but there appears to be no consistent difference in composition referable to the size of the leaf. As is well known, the size of apparently normal orange leaves may vary widely. Even on a given tree, the fully mature leaves of certain cycles of growth may be at least twice as large as others.

From the analysis of many other samples in this laboratory it may be said that the composition of mature orange leaves when grown in California is remarkably uniform, provided, however, that the leaves be borne on vigorous trees. On the other hand, the composition of the leaves of improperly nourished and diseased trees may vary widely. If the supply of available nitrate be deficient, the content of nitrogen in the leaves may be considerably below that reported above, but there seems to be some doubt whether the reverse is true.

COMPOSITION OF LEMON AND GRAPEFRUIT LEAVES

The analysis of mature Eureka lemon and Marsh seedless grapefruit leaves is submitted in Tables III and IV.

Two of the samples of lemon leaves were collected in midwinter and the other on August 29. They were grown on widely different types of soil. The Riverside sample grew on sandy loam, the Whittier sample on heavy adobe, and the Tustin sample on highly calcareous sandy loam soil. The grapefruit leaves were grown on sandy loam.

The composition of the different samples of lemon leaves is fairly uniform, the average being similar to the average composition of Valencia orange leaves. On the other hand, the composition of the grapefruit leaves closely resembles that of Navel orange leaves.

The composition of the leaves of the different varieties and species of citrus has been found to be remarkably uniform from the standpoint of both the ash and the dry matter. A more detailed discussion of the composition will be given below.

TABLE III.—*Composition of mature lemon and grapefruit leaves*

LEMON

Locality.	Date collected.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Riverside, plot U	Jan. 30, 1917	3.07	0.08	32.46	1.73	3.96	0.67	2.13	2.48	0.15
Whittier	Feb. 1, 1917	1.21	.07	34.02	2.55	4.63	.28	3.27	3.82	.22
Tustin	Aug. 29, 1917	1.02	.07	33.16	1.44	6.00	.30	2.12	2.43	.16
Average		1.77	.07	33.21	1.91	4.86	.42	2.51	2.91	.18
GRAPEFRUIT										
Riverside	Dec. 10, 1917	2.18	0.09	31.25	2.31	5.96	0.25	3.21	2.99	0.12

TABLE IV.—*Composition of mature lemon and grapefruit leaves*

LEMON

Locality.	Date collected.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
				SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
		Per cent.	*Per cent.*										
Riverside, plot U	Jan. 30, 1917	58.35	18.21	0.56	0.014	5.91	0.32	0.72	0.12	0.16	0.29	2.10	0.03
Whittier	Feb. 1, 1917	56.67	15.12	.18	.010	5.14	.39	.71	.04	.20	.38	2.65	.03
Tustin	Aug. 8, 1917	61.42	17.67	.18	.012	5.86	.25	1.06	.05	.16	.29	2.28	.03
Average		58.81	17.00	.31	.012	5.64	.32	.83	.07	.17	.32	2.34	.03
GRAPEFRUIT													
Riverside	Dec. 10, 1917	59.39	17.65	0.38	0.016	5.51	0.41	1.06	0.04	0.21	0.22	1.98	0.02

COMPOSITION OF ORANGE LEAVES AT DIFFERENT STAGES OF GROWTH

The results obtained from the analysis of samples of leaves approximately one month of age, gathered on May 11, 1917, were found to be considerably different from previous analyses of mature leaves. Samples representing the new spring growth and that of the previous year, gathered from the same trees on May 21, 1917, also proved to be widely different in composition. These results, together with the discordance between the analyses previously made in this laboratory and those published by Blair (*2*) from Florida and by Jensen (*7*) from California, suggested the desirability of making a study on the composition of orange leaves at different stages of growth.

Samples were collected at four different intervals in the growth cycle. The first represented leaves approximately 1 week old; the second, those 6 to 8 weeks old; the third, leaves at full maturity, the ages of which ranged from 6 months to approximately 2 years; the fourth, old leaves that were about to be shed, as indicated by their yellowish brown color. Each sample was picked from six normal, vigorously growing trees of plot V at the Citrus Experiment Station, Riverside, Calif. The samples representing different ages were all taken from the same trees, and those representing the first three periods of growth were gathered on the same day, November 9, 1917. These trees support an abundant foliage; and, as frequently occurs, they at that time bore numerous shoots of varying ages, ranging from a few days to 2 or more years of age, which made it possible to secure samples of widely different ages on a given day. The samples of old leaves were gathered December 10, 1917.

The data expressed as percentages of the ash show that notable changes take place in the relations of certain constituents as growth proceeds. Especially prominent among these changes are the decreases in the percentages of phosphate and potassium, on the one hand, and the increases in calcium on the other. For example, the ash of navel leaves at the age of 1 week was found to contain 16.83 per cent phosphate (PO_4), at 6 weeks 7.10 per cent, at maturity 2.47 per cent, while the ash of old leaves contained only 1.32 per cent.

The changes in the percentages of potassium were quite parallel to those of phosphate. When navel leaves were 1 week of age, the ash contained 19.87 per cent potassium, when 6 weeks of age, 10.32 per cent, when mature, 5.68 per cent, while the old leaves contained only 1.66 per cent.

The percentages of calcium underwent changes quite opposite to those of potassium. With the ash containing 20.72 per cent calcium when the leaves were 1 week old there was an increase to 28.44 per cent at 6 weeks, to 33.21 per cent at maturity, and finally to 34.41 per cent in the very old stage.

TABLE V.—*Composition of orange leaves at different stages of growth*

NAVEL

Age of leaves.	Constituents of ash.								
	SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1 week	1.82	0.28	20.72	3.91	19.87	1.20	16.83	5.88	0.32
6 weeks	.87	.17	28.44	3.29	10.32	.57	7.10	5.14	.15
Mature, 6 months to 2 years	1.70	.12	33.21	2.20	5.68	.46	2.47	3.51	.27
Very old, 3 or more years	1.96	.07	34.41	1.67	1.66	.44	1.32	3.09	.40
VALENCIA									
1 week	2.42	0.24	19.82	3.49	19.45	1.35	14.28	5.30	1.04
6 weeks	1.10	.14	27.24	2.89	12.66	.74	7.44	4.42	.58
Mature, 6 months to 2 years	1.13	.13	32.99	2.08	5.93	.58	2.79	3.22	.78
Very old, 3 or more years	1.11	.07	34.49	1.71	1.88	.42	1.49	1.94	1.31

TABLE VI.—*Composition of orange leaves at different stages of growth*

NAVEL

Age of leaves.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
			SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
	Per cent.	*Per cent.*										
1 week	72.31	6.54	0.12	0.018	1.36	0.26	1.30	0.08	0.36	0.25	3.01	0.02
6 weeks	70.81	9.20	.08	.016	2.62	.30	.95	.05	.21	.27	2.45	.01
Mature, 6 months to 2 years	60.98	16.95	.29	.021	5.63	.37	.96	.08	.13	.36	2.39	.05
Very old, 3 or more years	60.73	21.39	.42	.016	7.36	.36	.36	.09	.14	.37	1.31	.08
VALENCIA												
1 week	72.69	7.53	0.18	0.018	1.49	0.26	1.46	0.10	0.35	0.25	3.03	0.08
6 weeks	68.54	9.12	.10	.013	2.48	.26	1.16	.07	.22	.28	2.53	.05
Mature, 6 months to 2 years	60.52	16.35	.18	.021	5.39	.34	.97	.09	.15	.28	2.37	.13
Very old, 3 or more years	59.10	22.32	.25	.016	7.70	.38	.42	.09	.13	.30	1.35	.29

Among the other necessary nutrients, the percentages of iron, magnesium, and sulphate decreased with age, although to a lesser degree than potassium and phosphate. The ash of the youngest leaves contained approximately twice as much iron as that of the mature leaves, and differences almost as great occurred in the percentages of magnesium and sulphate.

As was anticipated, the changes that take place in Valencia orange leaves are quite similar to those of navel leaves.

The percentages of phosphorus and sulphur refer to the total amounts as determined by the magnesium-nitrate and sodium-peroxid fusion methods, respectively, and are somewhat higher than the corresponding data calculated from the ash analyses. As is well known, organic materials usually lose a portion of their phosphorus and sulphur in the ashing process.

It will be noted that the content of water decreased considerably as growth took place. At 1 week of age the navel leaves contained 72.31 per cent water, at 6 weeks 70.81 per cent, at maturity 60.98 per cent, and the very old leaves still contained 60.73 per cent. The content of total ash, on the other hand, increased markedly with age, rising from 6.54 per cent of the dry matter at the age of 1 week to the very high content of 21.39 per cent in the old leaves.

The nitrogen decreased from 3.01 per cent at the age of 1 week to 2.39 per cent at maturity, and finally to 1.31 per cent in the old stage. The percentage of phosphorus decreased still more rapidly during the actively growing period, but later the phosphorus content remained approximately constant. The percentage of potassium also decreased rapidly during the early period of growth but remained almost constant after the second period until the period of senility approached, when a still further decrease took place.

The percentage of iron in the dry matter was found to be reasonably constant at all stages of growth. However, in considering the iron content of these and all other samples reported herein, it is important to bear in mind that the analytical error involved in the determination of small amounts of this element is likely to be relatively great. For this reason small variations in the results are probably not significant. The percentages of sulphur and magnesium each increased somewhat as growth took place.

The constituent of the dry matter of orange leaves that undergoes the greatest percentage change as a result of growth is calcium. At 1 week of age, the navel leaves contained 1.36 per cent calcium, at 6 weeks 2.62 per cent, at maturity 5.63 per cent, and the very old leaves contained 7.36 per cent.

Of the supposedly unessential constituents, the greatest concentration of sodium was found in the young leaves; but the amount was always small, while the data for silica and chlorin show no consistent variation.

It is interesting to note that in certain respects the composition of orange leaves changes with growth, somewhat as is the case with the vegetative portion of other plants. With certain cereals a considerable portion of the potassium, magnesium, phosphorus, and nitrogen migrate from the leaves into other parts of the plant as maturity approaches (*9*, *10*). The potassium tends to accumulate in the straw of rice, while the magnesium, phosphorus, and nitrogen are translocated to the grain.

The composition of citrus leaves differs markedly from that of cereals in certain other respects. The ash content of the former increases much more rapidly and reaches a very high point in the old leaves. The calcium content increases very rapidly during the most actively growing period and continues to be deposited in the leaves, although at a somewhat slower rate, almost until the time the leaves fall off.

While it is probable that the composition of normal orange leaves varies to some extent when grown in different parts of the world or on different soils in a given locality, careful study of the analyses of the Florida-grown leaves published by Blair (*2*) and those reported from Italy by Olivieri and Guerrieri (*13*) suggests that these were immature leaves. From Jensen's results (*7*), it is evident that his samples were not composed of mature leaves. Recognition of the relationships between the age and the composition of orange leaves is especially important in the study of the composition of mottled leaves, as will be pointed out more fully later.

It does not necessarily follow from the preceding discussion that a portion of a given element, potassium, for example, migrates back into other parts of the tree after the leaves reach a certain stage of development. Increase in the size of a leaf, owing to the elaboration of carbonaceous matter, may dilute the nutrients present and, therefore, lower the percentage without there being an actual loss. To establish this point, it is necessary to determine the weights of the constituents present per leaf at different periods. From the average weights of the individual leaves at each period we have calculated the content of the different constituents, expressing the results in grams per 1,000 leaves. (Table VII.)

The old Navel leaves were considerably smaller on the average than either those representing maturity or 6 weeks of age, while the mature Valencia leaves were larger than the old leaves of the same variety. In addition, the leaves of each sample of the Valencia variety were considerably larger than the corresponding Navel leaves.

Despite these irregularities in the size of the leaves, the data show that the content of calcium in a given orange leaf increases very rapidly during the early part of the growth period. In the Navel leaves, approximately a tenfold increase in calcium content took place between the first and the sixth week of age. From the sixth week to maturity a further increase, more than twofold, took place, and finally the calcium content increased still further as the leaves approached the time of normal dropping

TABLE VII.—*Average amounts of constituents in 1,000 normal orange leaves*

Age of leaves.	Fresh material.	Dry matter.	Ca.	Mg.	K.	P.	N.	S.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
NAVEL								
1 week	227	62.80	0.85	0.16	0.82	0.23	1.89	0.16
6 weeks	1,094	319.30	8.36	.96	3.03	.67	7.82	.86
Mature	852	322.03	18.13	1.19	3.09	.42	7.70	1.16
Very old	756	297.00	21.87	1.07	1.07	.41	3.89	1.10
VALENCIA								
1 week	356	97.30	1.45	0.25	1.42	0.34	2.94	0.24
6 weeks	1,183	372.22	9.23	.97	4.32	.82	9.42	1.04
Mature	1,080	426.29	22.98	1.44	4.13	.64	10.10	1.19
Very old	801	327.70	25.23	1.24	1.38	.43	4.42	.98

The rates of increase in magnesium and sulphur are also rapid during the early part of the growth period, and each of these constituents continues to accumulate in the leaves up to maturity, but the absolute amounts never become high. Since irregularities occurred in the size of the leaves, it is doubtful whether any important amount of either magnesium or sulphur is translocated to other portions of the tree after maturity has been reached.

The maximum amounts of potassium, phosphorus, and nitrogen were deposited before the leaves were 6 weeks of age. The rates of increase of each were considerably less than that of calcium. The data show that a considerable portion of these elements migrates away from the leaves after certain periods. With potassium and nitrogen the loss takes place after maturity has been reached, while the phosphorus begins to recede even before maturity is attained.

Similar data for iron are omitted because of the magnitude of the analytical error involved in its determination.

Samples representing more frequent intervals in the growth cycle would certainly afford more detailed information regarding absorption. It is possible that the analysis of such samples when plotted might show breaks in the curves not indicated by the existing data. For example, the exact period in the growth cycle when the leaves contained the maximum amount of potassium might be shifted to some extent and other fluctuations might also be found. However, other analyses of immature orange leaves at different seasons of the year show a fairly close agreement with those reported above. On the whole, we are inclined to believe that the main features of the composition of the orange leaf have been determined.

It seems appropriate to emphasize the fact that citrus leaves are extremely calcareous, and much more so than most of the economic plants. As is well known, the ash of some of the legumes contains high percentages of calcium, but relatively few have been reported to contain as high percentages of calcium as citrus leaves. Not only is the ash of citrus leaves high in calcium but the total ash content is high also. It is unusual to find dried plant material that contains from 5 to 7 per cent calcium.

COMPOSITION OF MOTTLED ORANGE LEAVES

The condition of citrus trees known as mottle-leaf has been widely discussed. Much study has already been devoted to it, and several hypotheses have been advanced concerning the disease. The symptoms, mode of occurrence, and general distribution were fully discussed in a paper by Briggs, Jensen, and McLane (*3*). The disease is commonly thought to result from some nutritional disturbance, but the cause has not been definitely determined.

TABLE VIII.—*Composition of mottled orange leaves*

NAVEL

Locality.	Date collected.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Riverside, plot H	Mar. 19, 1915	3.10	0.16	24.27	2.04	13.13	1.92	4.91	4.86	0.26
Riverside, plot A	Dec. 9, 1916	1.73	.16	23.03	2.12	15.87	1.04	6.44	4.93	.31
Riverside, plot H	do	1.44	.14	23.38	2.42	13.40	1.65	7.16	4.42	.34
Do	May 21, 1917	1.80	.13	26.24	2.47	12.22	.70	5.56	4.74	.67
Average		2.02	.15	24.23	2.26	13.65	1.33	6.02	4.74	.39
VALENCIA										
Riverside, plot A	June 13, 1916	4.07	0.14	27.76	2.40	10.22	0.90	4.47	4.72	0.61
Riverside, plot H	do	3.74	.12	29.33	2.29	8.11	.63	4.43	4.51	.39
Arlington	Jan. 29, 1917	2.15	.13	25.77	3.70	13.81	.81	6.89	4.40	.24
Riverside, plot H	May 23, 1917	1.84	.10	28.37	2.54	9.22	.65	5.27	3.98	.61
Average		2.95	.12	27.81	2.73	10.34	.75	5.26	4.40	.46

TABLE IX.—*Composition of mottled orange leaves*

NAVEL

Location.	Date collected.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
				SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
		Per cent.	*Per cent.*										
Riverside, plot H	Mar. 19, 1915	63.75	15.15	0.47	0.025	3.68	0.31	1.99	0.29	0.29	0.37	3.19	0.04
Riverside, plot A	Dec. 9, 1916	64.84	12.36	.21	.020	2.85	.26	1.96	.13	.25	.46	3.12	.04
Riverside, plot H	do	63.57	13.64	.20	.019	3.19	.33	1.83	.22	.33	.50	3.60	.05
Do	May 21, 1917	64.22	14.80	.27	.019	3.88	.37	1.81	.10	.28	.36	3.91	.10
Average		64.34	13.99	.29	.021	3.40	.32	1.90	.18	.29	.42	3.45	.06
VALENCIA													
Riverside, plot A	June 13, 1916	62.16	13.94	0.57	.....	3.87	0.33	1.42	0.13	0.17	0.38	2.59	0.06
Riverside, plot H	do	62.50	15.98	.60	0.019	4.69	.36	1.29	.10	.25	.37	3.03	.06
Arlington	Jan. 29, 1917	66.83	11.70	.25	.015	3.01	.22	1.62	.09	.25	.30	3.21	.09
Riverside, plot H	May 23, 1917	62.87	15.07	.28	.014	4.27	.38	1.39	.10	.28	.33	3.57	.08
Average		63.59	14.17	.45	.016	3.96	.32	1.43	.10	.24	.34	3.10	.07

We have analyzed different portions of orange and lemon trees affected with mottle-leaf, as well as grapefruit leaves and samples representing different degrees of mottling. Most of the samples were collected from the fertilizer plots of the Citrus Experiment Station. In all cases the leaves were collected from shoots 6 or more months of age. The analysis of orange leaves in an advanced stage of mottling is presented in Tables VIII and IX.

Comparison of the data with the previously submitted analyses shows at once that the composition of mottled leaves differs considerably from that of average mature normal leaves. The principal differences are found in the greater percentages of potassium and phosphate, on the one hand, and the lesser percentages of calcium on the other. The ash of mottled leaves also contains greater percentages of magnesium and sulphate, while the iron, silica, sodium, and chlorin do not differ materially.

Considerable variations will also be noted among the different samples of mottled leaves. This is probably due to the varying degrees of mottling represented by the samples. However, every sample of mottled leaves that has been analyzed in this laboratory has been found to vary from the normal in the same general direction.

The average content of water in mottled leaves was found to be slightly higher than in normal leaves and the ash content somewhat lower. Considering the dry matter, the most pronounced differences are found in the lesser calcium content, on the one hand, and the abnormally high percentages of potassium and phosphorus in mottled leaves, on the other. The average nitrogen content of mottled leaves is also considerably above normal, as was previously pointed out by McBeth (*11*).

From his analyses of normal and mottled citrus leaves, Jensen (7) failed to find any consistent difference in composition. In order to insure uniformity in the age of his samples, he collected the leaves from the current season's growth. On the dates two of his samples were collected, April 18 and May 11, the current season's growth is probably never mature at Riverside. Furthermore, the calcium content, which he reported, was very much below that of any mature normal orange leaf we have been able to find. It seems safe to conclude, therefore, that Jensen's studies were made with immature leaves. It is possible, of course, that the variations in composition incident to mottling may not occur until after the leaves have reached a certain stage of growth, although recent analysis of a sample of leaves about 10 days of age, taken from severely mottled trees, indicates that the composition may begin to diverge from the normal at a very early period.

It is well known that, with the exception of severe cases of mottle-leaf, the discoloration ordinarily does not become apparent until the leaves have reached an age of 2 to 3 months. Subsequently, the degree of discoloration becomes increasingly intense until the period of normal

maturity. In addition, mottle-leaf is usually most pronounced from September to February, when it becomes very noticeable on the leaves of the previous spring and summer cycles of growth.[1]

Some light may be thrown on mottle-leaf by comparing the composition of mottled leaves with that of normal leaves at different stages of growth. By reference to Tables V and VIII it will be seen that the composition of the ash of the former is quite similar to that of normal leaves approximately 6 weeks of age, although the total ash content of mottled leaves is considerably higher (compare Tables VI and IX). It is especially interesting to note that the nitrogen content of mottled leaves is somewhat higher than that of normal leaves at the age of 1 week and much greater than that of normal leaves at the age of 6 weeks.

The data indicate, therefore, that the essential nutrients are deposited in mottled orange leaves at abnormal rates. A satisfactory explanation of this fact can not now be given. The rising sap is itself probably abnormal in composition.

By calculating the weights of the several constituents contained in a unit number of mottled leaves, it is found (Table X) that notwithstanding the fact that the average size of the mottled leaves was less than one-half that of normal leaves they contained as great amounts of potassium and approximately as much phosphorus per leaf (compare Tables VII and X). On the other hand, the content of calcium was less than one-third as great as normally occurs, while the magnesium, sulphur, and nitrogen were intermediate in amount.[2]

The preceding analyses represent extreme cases of mottling. Samples of Valencia orange leaves at a less advanced stage have also been studied. These latter were of an intermediate size, showing the typical yellowish spots between the veins. They were selected from trees a considerable portion of whose foliage was normal and some of which bore a fair crop of fruit. The results are recorded in Table XI

The percentages of calcium and potassium closely approach those of severely mottled Valencia leaves (Tables VIII and IX), but the phosphorus content is more nearly normal. The percentage of nitrogen was found to be no greater than occurs in normal Valencia leaves.

Thus, it appears that the early stages of mottling are first attended by the absorption of subnormal amounts of calcium [3] and supernormal amounts of potassium and phosphorus, and that modifications in the absorption of nitrogen occur later.

[1] Mottled leaves fall off in large numbers during the latter part of the winter and early spring. New shoots developing at this season give the trees the appearance of having recovered from the disease. These latter, however, may become mottled the following fall. It is never safe to pass judgment on the state of the disease in the spring or early summer. We have never known of a leaf once severely mottled which became normal later. New leaves grown later, however, may be entirely normal.

[2] These data were calculated for only a portion of the samples of mottled leaves, because the average weight of the leaves was not determined for all the samples.

[3] Jensen (7) found that the yellow spots of mottled orange leaves, similar to those discussed here, contain less calcium than the remaining portion of the leaf.

TABLE X.—*Average amounts of constituents in 1,000 mottled orange leaves*

NAVEL

Locality.	Fresh material.	Dry matter.	Ca.	Mg.	K.	P.	N.	S.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Riverside, plot A	480	169	4.81	0.44	3.31	0.42	5.27	0.78
Riverside, plot H	521	189	6.03	.62	3.46	.62	6.80	.94
Riverside, plot H	425	148	5.74	.55	2.68	.31	5.78	.53
Average	475	169	5.53	.54	3.15	.45	5.85	.75

VALENCIA

Arlington	755	250	7.52	0.55	4.05	0.62	8.02	0.75
Riverside, plot H	352	130	5.55	.49	1.90	.36	4.64	.43
Average	553	190	6.53	.52	2.97	.49	6.33	.59

TABLE XI.—*Composition of Valencia orange leaves at intermediate stages of mottling*

Locality.	Date.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per nt.*	*Per cent.*	*Per cent.*	*Per cent.*
Escondido	Feb. 1, 1917	2.67	0.13	28.74	2.13	11.38	0.82	4.18	2.80	0.44
Riverside, plot R	May 29, 1917	2.83	.06	30.55	2.00	7.48	1.18	3.23	3.74	.40
Riverside, plot U	June 13, 1916	.....	.....	28.78	1.91	9.16	.90	6.44	4.26	.26
Average		.....	.....	29.36	2.01	9.34	.97	4.62	3.60	.37

Locality.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
			SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
	Per cent.	*Per cent.*										
Escondido	60.72	13.58	0.36	0.018	3.90	0.14	1.54	0.11	0.17	0.27	2.53	0.06
Riverside, plot R	60.47	14.12	.40	.009	4.31	.28	1.05	.17	.18	.32	1.77	.06
Riverside, plot U	62.07	13.51	.55	.....	3.89	.41	1.24	.12	.22	.32	2.36	.03
Average	61.09	13.74	.44	.....	4.03	.28	1.28	.13	.19	.30	2.22	.05

Severely mottled lemon and grapefruit leaves have also been analyzed (Tables XII and XIII).

The results show that the composition of mottled lemon and grapefruit leaves is similar to that of mottled orange leaves. As was found from the analysis of normal leaves, the composition of lemon leaves closely resembles that of Valencia orange leaves, while the composition of grapefruit leaves was found to be like that of Navel leaves. However, the different varieties and species do not vary greatly in composition.

The fact that the composition of the leaves of one species of citrus is affected in the same general way as that of other species is not surprising, since their appearance when mottled is also similar.

As is well known, it is rare that all the leaves on a given orange tree are mottled. As a rule, those growing on the outer portions of the tree are the most severely affected, as sometimes, although not invariably, is the case with the leaves borne on the south and southeastern portion of the trees. The leaves of severely affected trees, however, may be mottled throughout the tree. Frequently the greater portion of the leaves borne by the shoots of a given growth cycle may be mottled, while those immediately preceding and following this cycle may be entirely normal in appearance. It is interesting, therefore, to compare the composition of normal and mottled leaves from the same tree.

With this end in view, samples of normal-appearing leaves were collected from the same trees from which some of the previously discussed samples of mottled leaves were drawn and on the same days. The analyses are reported in Tables XIV and XV.

The data are concordant with the previously reported analyses of normal leaves (Tables I and II). The results suggest that the leaves of different cycles of growth are mutually independent in composition and that the peculiarities in the composition of mottled leaves are not due to any special peculiarity of the tree upon which they have grown. A leaf of normal appearance borne by an orange tree the major portion of whose foliage is severely mottled, as were some of these samples, has approximately the same composition as any other normal orange leaf.

Some study has also been devoted to citrus trees affected by chlorosis[1] and injured by alkali, the results of which will be presented elsewhere.

The composition of albino and etiolated plants is of interest in this connection. Church (*4*, *5*) analyzed the normally green and albino portions of the maple (*Acer negundo*), holly (*Ilex aquifolium*), ivy (*Hedera helix*), and several other species. He found that the albino portions uniformly contained greater amounts of water than the green portions. The ash of the former contained greater amounts of potash and phosphoric acid and lesser amounts of lime than the latter, while the content of iron was approximately the same.

[1] Chlorosis of citrus, as it occurs in California, is distinguishable from mottle-leaf by a general fading of the chlorophyl over the entire mesophyl tissue, while mottle-leaf, as the name implies, denotes the lack of chlorophyl in spots between the veins.

TABLE XII.—*Composition of mottled lemon and grapefruit leaves*

LEMON

Locality.	Date collected.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Riverside, plot G	Nov. 4, 1917	2.31	0.26	26.94	3.10	11.64	1.56	4.82	5.02	0.27
Arlington	Dec. 10, 1917	1.77	.09	25.15	2.39	15.00	.21	5.33	4.50	.36
Average		2.04	.17	26.04	2.74	13.32	.88	5.07	4.76	.31

GRAPEFRUIT

Arlington	Dec. 10, 1917	1.72	0.08	24.28	2.57	15.31	0.29	5.63	3.95	0.29

TABLE XIII.—*Composition of mottled lemon and grapefruit leaves*

LEMON

Locality.	Date. collected.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
				SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
		Per cent.	*Per cent.*										
Riverside, plot G	Nov. 4, 1917	67.82	13.99	0.32	0.037	3.77	0.43	1.63	0.22	0.25	0.34	2.72	0.04
Arlington	Dec. 10, 1917	65.05	13.85	.24	.013	3.84	.33	2.08	.03	.26	.36	2.40	.05
Average		66.88	13.92	.28	.025	3.80	.38	1.85	.12	.25	.35	2.56	.04

GRAPEFRUIT

Arlington	Dec. 10, 1917	64.83	16.09	0.28	0.013	3.91	0.41	2.46	0.05	0.32	0.33	2.86	0.05

TABLE XIV.—*Composition of normal orange leaves from trees bearing many mottled leaves*

NAVEL

Locality.	Date collected.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Riverside, plot A	Dec. 9, 1916	2.88	0.16	30.77	1.46	7.49	0.98	2.44	3.94	0.36
Riverside, plot H	do	2.34	.15	31.71	2.07	5.94	.96	2.49	2.89	.57
Average		2.61	.15	31.24	1.76	6.71	.97	2.46	3.41	.46
VALENCIA										
Riverside, plot A	June 13, 1916	(a)	(a)	31.77	1.56	4.72	0.55	1.97	2.79	0.36
Riverside, plot H	do	(a)	(a)	33.76	1.68	3.01	.56	1.77	2.38	.17
Riverside, plot U	do	(a)	(a)	32.77	1.73	4.35	.52	1.35	2.50	.29
Average				32.77	1.66	4.06	.54	1.70	2.56	.27

[a] Not reported; results abnormally high because samples were contaminated with particles of dust.

TABLE XV.—*Composition of normal orange leaves from trees bearing many mottled leaves*

NAVEL

Locality.	Date collected.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
				SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
		Per cent.	*Per cent.*										
Riverside, plot A	Dec. 9, 1916	58.52	16.44	0.47	0.026	5.06	0.24	1.23	0.16	0.13	0.37	2.52	0.06
Riverside, plot H	do	55.59	17.52	.41	.027	5.56	.36	1.04	.17	.15	.37	2.60	.10
Average		57.05	16.98	.44	.026	5.31	.30	1.13	.16	.14	.37	2.56	.08
VALENCIA													
Riverside, plot A	June 13, 1916	57.63	17.32	(a)	(a)	5.50	0.27	0.82	0.09	0.12	0.29	2.23	0.06
Riverside, plot H	do	55.71	20.23	(a)	(a)	6.83	.36	.63	.11	.15	.34	2.35	.03
Riverside, plot U	do	58.33	18.05	(a)	(a)	5.91	.31	.78	.09	.14	.22	1.92	.05
Average		57.22	18.53			6.08	.31	.74	.10	.14	.28	2.17	.05

[a] Not reported.

Palladin (*14*) also found that the composition of the normal green and etiolated specimens of *Vicia faba*, the latter having been grown in the absence of light, differed in composition in the same general way as the normal and albino plants reported by Church. Weber (*16*) studied the effects of different parts of the spectrum on the composition of plants and found similar effects. Jensen (7) has recorded similar observations on the leaves of the privet plant, *Ligustrum aurea*.

While the fundamental cause of vegetable albinism is not known, the fact that light of certain wave lengths is essential to the formation of chlorophyl is well known; but in mottled citrus leaves the deficiency of chlorophyl certainly can not be caused by an insufficiency of light.

The fact that the composition of albino and etiolated plants differs from that of normal specimens in the same general way as is the case with mottled and normal citrus leaves shows that different causes may bring about similar effects in different species of plants. This fact also suggests at once that the composition of a plant may not afford a safe basis for forming a judgment as to the cause of a particular phenomenon. A satisfactory elucidation of these questions is not possible at present owing, in part at least, to the lack of definite knowledge concerning the fundamental principles underlying the growth processes of plants. The formation of chlorophyl is undoubtedly the result of a number of interdependent factors, and it is highly probable that either the absence or the inhibition of any one of these factors may prevent the formation of chlorophyl or ultimately lead to its decomposition.

COMPOSITION OF THE SAP OF ORANGE LEAVES

Some study has also been devoted to the sap of orange leaves. The sap was obtained by first subjecting the leaves to a temperature a few degrees centigrade below zero for a period of several hours. Immediately after the leaves were removed from the freezing chamber they were quickly ground to a pulp with an ordinary meat grinder. The juice was then pressed from the pulp by the use of a hand-screw press. A portion of the juice was filtered through folded filter paper, and its specific gravity was determined by the pycnometer. Partial analysis was made on weighed portions of the juice by first evaporating to dryness and then using the methods previously described. Special investigations were also made on unfiltered portions of the sap as described below.

Mature normal leaves, collected from healthy navel orange trees on May 29, 1918, were first studied. A sample of 861 gm. of leaves yielded approximately 150 cc. of sap. Partial analysis gave the following results:

Specific gravity.	Ca.	K.	P.
1.08	*Per cent.* 1.07	*Per cent.* 0.54	*Per cent.* 0.036

These data show that the expressed sap of mature orange leaves is comparatively rich in solids, calcium, and potassium, but the ratio of calcium to potassium in the sap is widely different from the ratio of the total amounts of these elements in the leaf. (Table II.)

On June 5, 1918, three sets of samples of Valencia orange leaves were collected. One of these was composed of normal leaves about 6 weeks of age; another sample obtained from the same trees consisted of healthy mature leaves; whereas the third sample was chosen to represent severely mottled leaves of the previous year's growth. Each of the samples was divided into three parts, one of which was used to study the sap, another to determine the water-soluble constituents, and the third for total analysis.

The sap was pressed out after freezing as described above. The water-soluble constituents were extracted by first grinding 100 gm. of the fresh leaves in a meat grinder, shaking with 1,000 cc. distilled water for one hour, and filtering through filter paper. Total acidity was determined by titration with *N/10* sodium hydroxid, using phenolphthalein as indicator. It was necessary to dilute the sap considerably because of its dark color, and a high degree of accuracy is not claimed for the results. They are rather approximations. The acidity is expressed for convenience as anhydrous citric acid.[1] The results are presented in Tables XVI, XVII, and XVIII.

TABLE XVI.—*Composition of Valencia orange leaves at the age of 6 weeks*

	Specific gravity.	Ash.	Ca.	K.	P.	N.	Acid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Sap	1.065	3.17	0.67	0.72	0.045	.....	.....
Water extract [a]	1.005	.....	1.57	1.69	.13	.....	1.64
Total leaf [a]	.....	13.23	3.56	1.99	.21	2.45	.....

[a] Expressed in terms of dry matter.

TABLE XVII.—*Composition of normal mature Valencia leaves*

	Specific gravity.	Ash.	Ca.	K.	P.	N.	Acid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Sap	1.097	4.32	1.41	0.42	0.035	.....	.....
Water extract [a]	1.008	.....	2.85	.64	.063	.....	1.15
Total leaf [a]	.....	17.56	5.78	.94	.13	1.92	.....

[a] Expressed in terms of dry matter.

[1] The nature of the acid constituents of the leaves has not been investigated sufficiently to justify a definite statement as to their identity.

The results show that the sap of Valencia orange leaves at the age of 6 weeks contains smaller amounts of dissolved solids and total ash material than mature leaves. The calcium content increases more than twofold, and the potassium and phosphorus content decreases in passing to maturity. On the other hand, the sap of mottled leaves has a higher specific gravity and a higher ash content than that of mature normal leaves. The calcium content, however, is considerably less, while the potassium and phosphorus content is much higher.

It is evident from these data, therefore, that the sap of mottled Valencia orange leaves is materially different from that of normal leaves, either when they are 6 weeks of age or mature.

The water-soluble constituents were found to diverge in the same general direction as the sap. It is interesting to note that a very high percentage of the potassium, phosphorus, and calcium of orange leaves is soluble in water.

Samples of fully mature normal leaves and of severely mottled leaves of the previous year's growth were collected from Navel orange trees of the fertilizer plots at Riverside in August, 1918. The sap was expressed and used for more complete chemical study. (Tables XIX and XX.)

TABLE XVIII.—*Composition of mottled Valencia leaves*

	Specific gravity.	Ash.	Ca.	K.	P.	N.	Acid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Sap	1.118	4.85	1.13	0.91	0.111	.....	.....
Water extract [a]	1.009	.....	2.85	1.64	.180	.....	2.75
Total leaf [a]	.....	15.06	4.05	1.98	.243	3.00	.....

[a] Expressed in terms of dry matter.

The results are fairly concordant with those reported above for Valencia leaves. It is again shown that the composition of the sap of mottled orange leaves differs widely from that of normal leaves. The data also show that the ash of the sap of each sample contained considerably smaller percentages of calcium and higher percentages of iron than those reported above for the ash of the leaf as a whole, while the percentages of the other constituents are not materially different from those of the entire leaf. The calcium content of the sap of Navel orange leaves appears to be lower than that of Valencia leaves. (Compare Tables XVII and XX.)

Upon studying the preceding data, it seems difficult to escape the conclusion that there must be some important physiological significance attached to the fact that the sap of mottled orange leaves contains only about one-half as much calcium and approximately twice as much potassium and nitrogen and three times as much phosphorus as normal leaves.

TABLE XIX.—*Composition of the sap of mature normal and mottled Navel orange leaves*

Condition of leaves.	Specific gravity.	Constituents of sap, expressed as percentage of its ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
Normal	1.068	0.43	0.37	19.71	2.89	8.05	1.49	2.30	5.58	3.13
Mottled	1.074	.46	.36	9.88	2.30	17.32	.43	6.27	4.01	.82

TABLE XX.—*Composition of the sap of normal and mottled Navel orange leaves*

Condition of leaves.	Ash.	SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Normal	4.51	0.19	0.017	0.89	0.13	0.36	0.07	0.034	0.11	0.54	0.14
Mottled	4.89	.023	.018	.48	.10	.84	.02	.100	.09	.95	.04

The hydrogen-ion concentration of the sap was also determined by the use of the hydrogen electrode. Mature normal-leaf sap was found to give a P_H value of 5.816 and mottled-leaf sap a value of 5.647, which implies hydrogen-ion concentrations of 0.153×10^{-5} and 0.226×10^{-5}, respectively. These determinations are probably within the range of variation of different samples of the same leaves.

After the determination of the hydrogen-ion concentration, total acidity was determined by titration, using the hydrogen electrode to determine the end point. It was found that 10 cc. of the normal sap required 3 cc. *N/10* alkali and the mottled-leaf sap 7.05 cc. In other words, the actual acidity (hydrogen-ion concentration) of mottled-leaf sap is approximately the same as that of normal leaves. but the latter sap is more nearly saturated with base. It is probable that in each case the ionization of the acids is held at approximately the same level by the buffers present.

Samples of normal Navel orange leaves approximately one week of age, fully mature leaves, and severely mottled leaves of the previous year's growth were collected in April, 1919. The sap was expressed, and the hydrogen-ion concentration and total acidity were determined by the hydrogen electrode. Freezing-point depressions were also determined in portions of the unfiltered sap. The acidity is expressed in cubic centimeters of *N/10* sodium hydroxid required to neutralize 10 cc. of the sap.

TABLE XXI.—*Acidity and freezing-point depression of orange-leaf sap*

Condition of leaves.	P_H.	Hydrogen-ion concentration.	Total acidity.	Freezing-point depression.
			Cc.	*°C.*
Normal, 1 week old	6.069	0.852×10^{-6}	1.80	1.258
Normal, mature	5.664	$.217 \times 10^{-5}$	3.80	1.588
Mottled	5.647	$.226 \times 10^{-5}$	7.90	1.734
Mottled	5.630	$.235 \times 10^{-5}$	8.25	.....

These data show that the actual acidity (hydrogen-ion concentration) of mature orange-leaf sap is approximately two and one-half times as great as that of leaves at the age of 1 week; but again it is shown that the acidity of mottled leaves is approximately the same as that of normal leaves. The capacity to neutralize base—that is, total acidity—however, was fully twice as great in mature leaves as in those 1 week of age, while the mottled-leaf sap neutralized about twice as much base as the normal mature leaf sap.

The freezing-point depressions show that while the normal mature-leaf sap is more concentrated than that of young leaves the sap of mottled leaves is more concentrated than either.

The results of the preceding investigation on the sap of orange leaves are very suggestive. They are in harmony with the preceding ash

analyses in that they indicate that the composition changes materially as growth proceeds and that the composition of mottled leaves differs from that of normal leaves.

It is interesting to note that the total water content of mottled and normal mature leaves is roughly correlated with the concentration of the sap, but this correlation does not hold when immature leaves are compared with mature leaves.

GENERAL DISCUSSION

It has been shown that the composition of orange leaves changes rapidly as growth takes place. The relationships between the several constituents drawn from the soil undergo important alterations. The percentages of potassium and phosphorus, when expressed on the basis of either the ash or the dry matter, decline rapidly during the early part of the growth cycle and continue to decline, although at reduced rates, during the latter part of the growth period. The percentages of nitrogen in the dry matter also decrease as growth proceeds. The percentage of calcium, on the other hand, increases rapidly at first, and later more slowly. The concentration of iron is greatest in very young leaves, but later its concentration decreases slowly, while no very pronounced changes take place in the percentages of the other constituents. The concentration of the different constituents probably remains practically constant throughout the period of normal maturity.

As the leaves approach senility just preceding the time of normal dropping, notable amounts of potassium and nitrogen are translocated back into the stem or other portions of the tree. A part of the phosphorus also appears to leave the leaf sometime preceding the period of normal maturity. In contrast to certain cereals, the absolute content of magnesium does not decrease as maturity approaches.

It has been shown that a given orange leaf normally contains the maximum amounts of potassium, phosphorus, and nitrogen by the time it is approximately 6 weeks of age. It is interesting that the leaf also reaches its maximum size about the same time. On the other hand, the absolute content of calcium continues to increase until full maturity is reached.

Mature orange leaves are extremely rich in certain nutrients. The content of carbonated ash ranges from 14 to 18 per cent of the dry matter, and the nitrogen content is usually above 2 per cent. The most pronounced characteristic of the orange leaf, however, is found in its highly calcareous nature. When the leaf is mature, the dry matter contains from 5 to 6 per cent of calcium.

Lemon and grapefruit leaves are similar in composition to orange leaves.

The composition of mottled citrus leaves is widely different from that of normal leaves. The difference lies mainly in the smaller calcium content, on the one hand, and the greater content of potassium and

phosphorus, on the other. Usually the nitrogen content of mottled leaves is also abnormally high. The composition of mottled orange leaves resembles that of immature leaves, although the percentages of ash and nitrogen in the former are materially greater than in the latter.

It has been shown that the absolute amounts of potassium and phosphorus contained in mottled orange leaves are fully as great as ordinarily occur in normal leaves that are two or three times as large, while the calcium content is not more than one-third that occurring in average normal leaves.

The sap of normal orange leaves becomes increasingly concentrated and acidic as growth proceeds. When mature it is especially rich in calcium and contains fully twice as much of this element as of potassium.

The abnormalities of mottled leaves noted above also occur in the sap and among the water-soluble constituents. The sap of mottled leaves contains subnormal amounts of calcium and fully twice as high concentrations of potassium and phosphorus as mature normal leaves. The hydrogen-ion concentration of mottled leaves is not materially different from that of normal leaves, but the sap is less nearly saturated with base. In other words, abnormally large amounts of unionized acids occur in mottled-leaf sap.

Limited study of portions of citrus trees other than the leaves indicates that the composition of the leaf spurs of severely mottled trees varies from the normal in much the same way as the leaves. The composition of the older wood, however, is more nearly normal. On the other hand, both the large roots and small rootlets of severely mottled trees appear to contain considerably less potassium and phosphorus than normal roots, while the calcium content is approximately normal.

Should more extended study confirm these latter observations, it would seem that the excessive proportions of potassium and phosphorus occurring in mottled leaves may have been drawn, in part at least, from the supply normally stored in the roots.

The results of these investigations suggest that mottled citrus trees are deficient in calcium, but the cause of the subnormal content of calcium can not be definitely stated.

While we recognize that growing plants have the power, through selective absorption, of regulating their composition to a marked degree, and that a given variation in the composition of a plant does not necessarily reflect a corresponding deficiency in the nutrient medium, the above data suggest that the abnormalities in the composition of different parts of mottled citrus trees may be due, in part at least, to the inability of the tree to satisfy its normal calcium requirements at critical periods.

It is well known that manure and other forms of decaying organic matter exert an ameliorating effect on mottle-leaf. It is interesting in this connection that the concentration of soluble calcium in the soil

becomes materially increased as a result of the decomposition of such materials (*8*). On the other hand, the occurrence of heavily compacted layers of soil (plowsole) around the roots, especially when present immediately below the depth of cultivation, and of soils of low organic content (*3*) and low natural solubility afford conditions that are conducive to mottle-leaf. Where such conditions occur, it is possible that the supplies of those nutrients which are normally absorbed at relatively high rates may become inadequate. The nature and extent of the root system of citrus trees must also be considered in this connection. It is interesting that the absorbing roots of citrus trees are not provided with the usual root hairs. Consequently, they may possess less absorbing surface than is afforded by other plants that normally absorb relatively large amounts of nutrients. These and other related questions will be more fully discussed elsewhere.

The fact that mottle-leaf sometimes appears on trees that have been injured by alkali suggests the possibility that alterations in permeability occasioned by the presence of excessive concentrations of salts, or possibly toxic substances of other kinds in the soil moisture, may prevent the roots from taking up normal amounts of calcium.[1]

If we may judge from the composition of normal leaves, the calcium requirements during the period when mottle-leaf develops most pronouncedly are extremely heavy. The leaves at that stage normally absorb calcium at a high rate.

Just why subnormal concentrations of calcium accompanied by supernormal concentrations of potassium and phosphorus in the leaves should afford conditions that tend to limit chlorophyl production is not known, if indeed further investigations prove that such is the case. There may, of course, be no causal relationship between these facts, but rather each may be the result of causes not yet suggested.

It is recognized that calcium is not a normal constituent of chlorophyl. In addition, while iron is essential to the formation of chlorophyl yet does not enter into its final composition, we are not aware that a similar relationship exists between calcium and chlorophyl formation. Consequently, even though further study should prove that mottle-leaf can be produced as a result of an inadequate supply of available calcium, it is probable that the lack of chlorophyl and its disappearance from the localized areas of the leaves would be found to be indirect rather than direct effects of a shortage of calcium. In any event, whether the shortage of calcium or some other factor conditions the deficiency of chlorophyl, photosynthesis is doubtless reduced by the lack of chlorophyl.

With an adequate supply of nitrogen, phosphorus, and potassium present in the soil moisture, osmosis might bring about the absorption of

[1] As is well known, the occurrence of mottle-leaf is sometimes correlated with the species of root stock, but this phase of the subject has not been systematically investigated in California. Mr. H. Atherton Lee has called the writer's attention to his studies on this phase of mottle-leaf in the Philippine Islands.

greater or lesser amounts of them, despite the deficiency of chlorophyl in the leaves; but the reasons why excessive amounts of these elements accumulate in mottled citrus leaves are not clear. It seems probable that some physico-chemical principle not elucidated by the preceding data must be fundamentally involved.

Before any explanation of mottle-leaf can be safely accepted, it is necessary to show that the disease can be produced experimentally, and that too under conditions admitting of scientific analysis. Additional studies already projected may throw further light on this subject.

Whatever may ultimately be found to be the primary cause of mottle-leaf, the preceding investigations strongly suggest that the leaves are not suffering from inadequate supplies of potassium, phosphorus, or nitrogen. We have also found little, if any, indication of a deficiency of iron.

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CONTROL OF FLUKE DISEASES BY DESTRUCTION OF THE INTERMEDIATE HOST[1]

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Flukes have long been known as causative agents of disease in animals, especially sheep; in fact the loss resulting from their ravages in some sheep-raising countries can be estimated in millions of dollars annually. Within comparatively recent years flukes have been discovered to play an important rôle in some countries in the production of human disease. At present human fluke infections are known to be more or less prevalent in nearly all tropical and subtropical countries and in some countries of temperate climate. The blood flukes, Schistosoma, occur in the oriental countries and throughout most of Africa and tropical America. Human liver flukes, Clonorchis, and the lung flukes, Paragonimus, are primarily diseases of the Orient, but epidemic cases have been reported from other countries. The various species of intestinal flukes which are habitual or accidental human parasites occur in both Asia and Africa and probably in other tropical countries, but these are of minor importance.

The important relation of fluke infections to the public health in endemic countries is not generally realized. In Egypt, for instance, over half the population is said to suffer from schistosomiasis, and in an examination of 54 boys in the village of El Marg, near Cairo, Leiper (*10*) [2] found 49 to be infected. Cawston (*3*) states that in some districts in South Africa 80 per cent of the school boys and 10 per cent of the girls are infected and that Schistosoma infections seriously retard both the physical and mental development of the school children. Troops operating in endemic regions are much affected by the disease unless stringent preventive measures are taken. The British army suffered severely in the Boer war, and in 1914 the British Government was still under heavy expense for pensions for soldiers invalided by schistosomiasis. Laning, of the United States Navy, states that it is not uncommon for large proportions of the crews of patrol gunboats operating on the Yangtze River to be completely disabled by *Schistosoma japonicum* infections. Nakagawa (*13*) states that lung flukes are harbored by as high as 50 per cent of the population in some districts in Formosa, and in parts of Japan the infection is hardly less prevalent. Clonorchis, a human liver

[1] Contribution from the Zoological Laboratory, Oregon Agricultural Experiment Station, Corvallis, Oreg., and from the Biological Laboratory, Rice Institute, Houston, Tex.

[2] Reference is made by number (italic) to "Literature cited," p. 208.

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fluke, is even more prevalent in Japan and is said by Kobayashi (*9*) to affect as many as 60 per cent of the inhabitants of some endemic areas. Like malaria and hookworm disease, fluke diseases are comparatively seldom fatal in themselves but are particularly injurious in causing loss of efficiency, reduced vitality, and lowered resistance to other diseases. The long duration and relative incurability of fluke infections are a very serious factor. In this respect fluke infections are far more to be feared than are infections with intestinal parasites, most of which are relatively easy to expel. Of the numerous drugs which have been tried in the treatment of extra-intestinal fluke infections, only tartar emetic, recently shown by Christopherson (*4*, *5*, *6*, *7*) to be more or less specific in its action on Schistosoma, gives promise of being of any great value. No unquestionably effective remedy for lung or liver flukes has been found, and even the use of tartar emetic for schistosomiasis is far from satisfactory, since the serious symptoms of the disease are caused by the eggs of the worms deposited in the tissues and often continue to exist long after the worms are dead.

With regard to fluke diseases of domestic animals the situation is no less serious. The common liver fluke of sheep and cattle, *Fasciola hepatica*, is found almost all over the world in temperate climates, being prevalent wherever these domestic animals are grazed on wet or marshy pastures. In the British Isles, France, Germany, and other parts of Europe and in some parts of the United States, notably western Oregon and Washington and the humid districts of Texas, Louisiana, Florida, and other southern States, the losses from flukes in sheep, cattle, and goats amounts to millions of dollars annually, on account of loss of vitality among the animals, depreciation in quantity and quality of meat, and the loss of the infested livers themselves. In the Tropics Fasciola is largely replaced by other flukes—for example, the intestinal Amphistoma and Gastrodiscus, and various blood flukes, Schistosoma. As with human flukes, the extra-intestinal flukes of animals can not be reached readily by drugs, and as pointed out by Ransom and Hall (*16*) there is still much doubt about the efficacy of drugs which have been recommended for use against them, though there is room for much more experimentation.

On account of the difficulty encountered in treating or curing fluke diseases, preventive measures loom up with even greater importance than they do in dealing with hookworm or other intestinal parasites. The working out of preventive measures based on scientific knowledge has only recently become possible, for, although the life history and mode of infection of the common liver fluke, *Fasciola hepatica*, of sheep and cattle have been well known for a number of decades, such knowledge of human flukes has been acquired only in the past three or four years. Leiper's work on Schistosoma in Egypt in 1915–16 (*10*), Kobayashi's work on Clonorchis in Japan in 1915 (*9*), and Nakagawa's work on

Paragonimus in Formosa in 1916 (*13*) have given a definite basis for preventive measures against all these parasites of man and of the related parasites of domestic animals.

In every case of fluke infection of man or domestic animals in which the life cycle of the parasite has been worked out it has been shown that fresh-water snails act as necessary intermediate hosts. It appears, therefore, that if some efficient and practical method of destroying the snails could be found, this would furnish a logical point of attack in the control of all fluke diseases. Other preventive measures are, of course, valuable also and could be used as supplementary measures—for example, the impounding of water before use for drinking or bathing as a preventive against Schistosoma infections, the discouragement of the habit of eating improperly cooked meat of crabs in the case of Paragonimus and of fish in the case of Clonorchis, and care in the disposal of feces and urine in all cases. The last, exclusive of individual mechanical protection against infection, is the only preventive measure that can be adopted against hookworm and many other intestinal parasites. To accomplish this in some warm countries where there has never existed anything approaching sanitation and where the very idea of sanitation is so strange and foreign to the habits of life and thought of the natives is well nigh impossible. The fact, therefore, that fluke infections may possibly be controlled by attack upon an intermediate host instead of by reliance upon the enforcement of sanitary regulations makes the ultimate eradication of these infections, in spite of their relative incurability, a matter of brighter prospect than is the case with many other verminous parasites.

Already a number of suggestions for the destruction of the snails which act as intermediate hosts of flukes have been made. Thomas (*18*) advised the extensive scattering of salt on pastures where sheep were known to become infected by flukes, and he commented on the absence of fluke infestations among sheep grazing on salt marshes. The effect of the salt, of course, was to destroy the snail, Limnaea, which acts as the intermediate host. Leiper (*10*) suggested the eradication of the disease in agricultural districts in Egypt by the intermittent flow of water in the irrigation ditches, the water being turned off for 15-day periods, thus drying up the ditches and destroying the snails by desiccation. Such a procedure is, of course, very limited in its application, and in view of the remarkable resistance which many snails have to drouth it is doubtful whether all the implicated species could be killed by this method even if it were feasible. Leiper suggested that ammonium sulphate be applied to pools which were inhabited by the intermediate hosts of Schistosoma. Lime has been recommended by a number of writers, particularly Japanese, as the cheapest and best method of destroying snails. One Japanese writer, Ando (*1*) states that 1 per cent lime water killed 6 of 10 snails in seven hours, and a 1 per cent solution of copper sulphate would kill them in six hours. It is obvious that none of the above methods of

exterminating snails would be practical on a large scale, either on account of the prohibitive cost or on account of the excessive amounts of the material used and consequent injury to the water for drinking, bathing, or irrigation purposes.

In the hope of finding some effective means of destroying disease-carrying fresh-water snails a series of experiments was undertaken by the writer. The original purpose of the investigation was to find a solution to the liver fluke problem among sheep and cattle raisers in the Willamette Valley of Oregon, but it was realized that if a means of controlling all fresh-water snails could be found, the results would be of infinitely greater value than the solution of the local problem, and the experiments were carried on with this in mind.[1]

It was obvious that any chemical which could be used on a large scale for the destruction of snails in ponds, marshes, or streams must not be toxic to man or domestic animals in the dilutions used and must not be expensive. An attempt, therefore, was made to find a cheap chemical substance, readily soluble in water, which would be destructive to snails in relatively weak solutions and which would not render water either injurious or unpalatable for man or domestic animals.

The chemicals which were selected for preliminary experiments, the dilutions which were made, and the results obtained are shown in Table I. The snails, *Limnaea (Galba) bulimoides*,[2] were immersed in each solution, using chemically pure salts and tap water, Corvallis tap water being unusually clear, pure, and soft. The sign − indicates no evident effect, ± slight noticeable effect in behavior, + distinct illness without complete prostration, + + complete prostration, and ⊕ death. It was found later that snails which were apparently dead would sometimes revive if placed in fresh, aerated water; therefore the results shown in this table are not absolutely dependable. They do, however, demonstrate beyond question one striking thing—the fact that copper salts have an extremely toxic effect on these snails, even in such great dilutions as one part to a million of water. Mercuric bichlorid is the only other salt experimented with which approaches the salts of copper in its toxicity to snails, but since it is evidently not so effective as copper, is more toxic to higher animals, and is more expensive, no further experiments with it were carried out.

The salts of copper being evidently the most promising substance with which to attack aquatic snails all subsequent work was concentrated on them. Experiments with various copper salts ($CuCl_2$, $CuSO_4$, $Cu[NO_3]_2$) were tried, and it was found that with equivalent concentrations of the Cu_{++} ion their toxicity was approximately the same. Copper sulphate,

[1] The writer has been unable to get access to the following paper: GERMAIN, L. DE L'EFFET DES POISONS MINÉRAUX SUR QUELQUES MOLLUSQUES TERRESTRES ET FLUVIATILES DE FRANCE. *In* Bul. Soc. Amis Sci. Nat. Rouen, s. 4, ann. 34, 1898, sem. 1, p. 71–78. 1899.

[2] Snails specifically named in this paper were kindly identified by Dr. H. A. Pilsbry, Dr. F. C. Baker, or Mr. Bryant Walker.

being the cheapest copper salt, was therefore selected for further experimentation.

Chemical.	Dilution.	1 hour.	4 hours.	8 hours.	24 hours.
As_2O_3	1 to 1,000,000	−	8 ±[a] / 2 −	±	−
$Ba(NO_3)_2$	1 to 100,000	−	−	−	−
$CaOCl_2$	1.3 available chlorin per 1,000,000.	−	−	−	−
$CaOCl_2$	2.6 available chlorin per 1,000,000.	−	−	−	−
$Ca(OH)_2$	1 to 10,000	−	−	−	−
$CuCl_2$	1 to 100,000	++	++	⊕	⊕
$CuSO_4$	1 to 100,000	++	++	⊕	⊕
$CuSO_4$	1 to 1,000,000	+	++	++	⊕
$HgCl_2$	1 to 1,000,000	±	++	++	7⊕ / 3+
NaCl	1 to 1,000	−	−	−	−
NaCN	1 to 100,000	−	−	−	−
NaCN	1 to 1,000,000	−	−	−	−
$(NH_4)_2SO_4$	1 to 100,000	−	−	−	−
$(NH_4)_2SO_4$	1 to 1,000,000	−	−	−	−
$Pb(CH_2COOH)_2$	1 to 100,000	−	±	6+ / 4−	2++ / 6+ / 2−
$Pb(CH_2COOH)_2$	1 to 1,000,000	−	−	±	±
$ZnCl_2$	1 to 1,000,000	−	−	−	−
$ZnSO_4$	1 to 1,000,000	−	−	−	±

[a] Figures beside symbols indicate number of snails out of the 10 used in the experiment.

The effect of copper salts on various kinds of organisms is extremely variable. Their highly toxic effect on algae, first demonstrated by Moore and Kellerman (*11*), is well known, and copper sulphate is extensively and successfully used in eliminating algae from ponds and reservoirs. Copper sulphate is effective against some algae in dilutions up to 1 part in 25,000,000 or more parts of water but is commonly used in the proportion of 1 part to from 1,000,000 to 3,000,000 parts of water. Its bactericidal action is less marked and varies greatly with temperature. At 20° C., in water relatively free from organic matter, all pathogenic bacteria are destroyed in 24 hours at a dilution of 1 part to 400,000 parts of water. Peters (*14*) showed that the concentration necessary to kill instantly certain protozoa was 12 to 60 × 10^{-8} gram molecular parts per cubic centimeter of water (about 3 to 15 parts per million). The toxic effect of copper on fungi is as striking as its effect on algae and is taken advantage of commercially in the use of Bordeaux mixture for spraying trees and vines.

Curiously enough the effect of copper salts on both higher plants and higher animals is in general far less toxic than it is on lower animals and plants. In dilute solutions copper sulphate has a stimulating action on the growth of many higher plants, having been tested particularly on various grains. In the animal series, copper salts are usually

harmless in the dilutions which are lethal to the single-celled organisms. Copper is, in fact, a normal constituent of their tissues and replaces iron in the blood of some invertebrates. Experiments by the writer, as well as by others, show that copper, 1 part per million, is not injurious, at least within 48 hours, to annelids, crustaceans, or aquatic insect larvae. Of vertebrate animals, fish are highly susceptible, various species being affected by 1 part of copper sulphate in from 500,000 to 10,000,000 parts of water. Amphibians are immune to dilutions of 1 to 1,000,000. Contrary to popular opinion, copper is not highly toxic to mammals and can, in fact, be taken by the mouth in considerable quantities without injury. Five to 10 gr. (0.32 to 0.64 gm.) can be taken as an emetic. Horses and cattle can take 3.9 to 7.7 gm. and sheep 1.3 to 2.6 gm. It is evident, therefore, that copper salts in high dilution have a selective effect on various organisms, being particularly destructive to single-celled organisms, certain molluscs, and fishes. For destroying aquatic snails, therefore, copper sulphate can be used in perfect safety so far as any possibility of injury to man or domestic animals from drinking or bathing is concerned, without injuring the water for irrigation purposes, and without destroying other higher organisms, except certain species of fish.

After it was found that very dilute solutions of copper salts are specifically toxic to *Limnaea (Galba) bulimoides*, experiments were carried out to determine their effect on other species of snails and also to ascertain as accurately as possible the effect of varying concentrations of the salts. In all of these experiments only chemically pure copper sulphate was used. Preliminary experiments showed that there was no appreciable difference in effect whether distilled water or the local tap water was used in the experiments; therefore the tap water was used except for making up the stock 0.1 per cent and 0.01 per cent solutions. All the local species of snails of which sufficient numbers could be obtained were tried. It was not practicable to experiment with the species of snails which have actually been incriminated as the intermediate hosts of important flukes of man and domestic animals, but a greater variety of snails than those which have been incriminated were used, including representatives or close allies of all the incriminated families and in some cases genera.

Of the species used, *Planorbis callioglyptus* belongs to the family Planorbidae, to which belong Bullinus, Planorbis, and Physopsis, intermediate hosts of *Schistosoma haematobium* and *S. mansoni;* Goniobasis, according to Pilsbry, is closely akin to Melania, intermediate host of Paragonimus, Metagonimus, and Clonorchis; Fluminicola belongs to the family Amnicolidae in common with Blanfordia, intermediate host of *Schistosoma japonicum;* several species of Limnaea serve as intermediate hosts for *Fasciola hepatica.*

Some difficulty was encountered in correctly reading the effect produced on the snails, and all earlier experiments had to be discarded.

It was found that snails which were prostrate and would not respond to stimuli, and were therefore apparently dead, would frequently revive after being placed in fresh, aerated water for from 12 to 24 hours. The only criterion for death which was used, therefore, was failure to revive within 24 hours after being placed in fresh water.

Experiments, using 10 snails of a species in one liter of the solution at approximately 18° to 20° C., were made as follows:

DILUTION.	SPECIES TESTED.
1 to 100,000	*Goniobasis plicifera, Limnaea bulimoides, Physa occidentalis.*
1 to 500,000	*Fluminicola fusca, Goniobasis plicifera, Physa occidentalis.*
1 to 1,000,000	*Ancylus caurinus, Fluminicola fusca, Goniobasis plicifera, Limnaea bulimoides, L. proxima rowelli, Physa nuttalli, P. occidentalis, Planorbis callioglyptus.*
1 to 1,500,000	*Fluminicola fusca, Goniobasis plicifera, Physa occidentalis.*
1 to 2,000,000	*Fluminicola fusca, Goniobasis plicifera, Limnaea bulimoides, L. proxima rowelli, Physa occidentalis, P. nutalli, Planorbis callioglyptus.*
1 to 2,500,000	*Fluminicola fusca, Goniobasis plicifera, Limnaea bulimoides, L. proxima rowelli, Physa occidentalis.*
1 to 3,000,000	*Fluminicola fusca, Goniobasis plicifera, Limnaea bulimoides, Physa occidentalis.*
1 to 4,000,000	*Physa occidentalis.*
1 to 5,000,000	*Fluminicola fusca, Goniobasis plicifera, Limnaea bulimoides, L. Proxima rowelli, Physa nuttalli, Physa occidentalis.*
1 to 10,000,000	*Limnaea bulimoides, Physa occidentalis.*

The results of these experiments may best be summarized as follows:

1. All species of snails experimented with, eight in all, belonging to six genera and as many families, are similar to each other in their susceptibility to copper sulphate. There is, in fact, much more individual variation shown than there is difference between species. Ancylus, Fluminicola, Limnaea, and Planorbis become prostrate a little more quickly than do Goniobasis and Physa. Goniobasis has a little more recuperative power than the other species after being placed in pure water.

2. All species die within 48 hours, many specimens sooner, in solutions of 1 to 500,000 and 1 to 1,000,000. Fluminicola, Limnaea, Physa, and Planorbis die within 48 hours in solutions of 1 to 1,500,000 and 1 to 2,000,000, but a few specimens of Goniobasis and one specimen of *Limnaea proxima rowelli* revived slightly after being placed in fresh water for 24 hours but died within 48 hours.

3. A 1 to 500,000 solution appeared to be no swifter in its action than was a solution of 1 to 2,000,000. In all dilutions between 1 to 500,000 and 1 to 2,000,000 some specimens revived after exposure for 24 hours, whereas after 48 hours none revived except as noted in the preceding paragraph.

4. Solutions ranging between 1 to 2,500,000 and 1 to 5,000,000 killed 50 per cent or more of the specimens, but not all, whereas all specimens revived after 48 hours in a 1 to 10,000,000 solution, although they became sick or prostrate while immersed in it.

5. In the 1 to 100,000 dilution, which was tried merely to ascertain whether this concentration would kill quickly, the snails became prostrate immediately upon being immersed and remained motionless, but they almost all revived after immersion for one hour.

The actual physiological effect of the copper salts on the snails has not been determined. Within a few minutes the snails immersed in a dilute copper-sulphate solution lie prostrate, being apparently unable to cling to the sides of the jar. A mucous albuminoid substance is exuded, and frequently feces, eggs, and even the penis, are extruded. It is highly probable that the poisoning effect is due at least in part to inactivation of enzyms necessary to life. Peters and Burres (*15*) showed that the concentrations of copper sulphate necessary to kill Paramoecium and Stentor were approximately the same as those necessary to inactivate their normal enzyms. It was thought that possibly there was a special tendency in snails to absorb copper, since this metal is an important constituent of the blood and is found only in minute traces in the normal environment. However, analyses of snails killed in copper-sulphate solutions, compared with normal snails, failed to show appreciably greater quantities of copper. Furthermore it was found that the snails succumbed as quickly in a few cubic centimeters of the solutions as they did in large quantities. Five specimens of *Limnaea bulimoides* were killed in 10 cc. of a 1 to 1,000,000 solution, yet the total amount of copper present was only about 0.0025 mgm., or 0.005 mgm. per snail. By analogy with *Helix pomatia*, which was shown by Dubois (*8*) to contain 6.11 mgm. of copper per 100 gr. of body weight, a specimen of Limnaea should normally contain several milligrams of copper. If the mode of action of the copper salts is by inactivation of enzyms, the similarity in effect of such varying dilutions as 1 to 500,000 and 1 to 2,000,000 is more readily explained.

The effect of a 1 to 1,000,000 copper-sulphate solution was also tried on the eggs of *Physa nuttalli* and of *Limnaea bulimoides*. Eggs in intact gelatinous masses were apparently uninjured by the copper solutions in 14 days, though the inclosed embryos seemed to grow more slowly than the controls.

There are a number of factors which influence the effect of copper sulphate on organisms in water, the most important being temperature, presence of algae, alkalinity, and organic matter in solution. As regards temperature, no extended experiments were carried out, but experiments with a 1 to 1,000,000 solution were carried out at temperatures of from 15° to 27° C., and the snails apparently succumbed as quickly at the lower as at the higher temperature. Water in which snails were to be destroyed would probably not fall below 15° C. in temperature. Alkalinity of water, to the extent normally found in natural ground waters, appears to have little effect on the action of the copper salts, although copper sulphate is precipitated as basic sulphates or carbonates in

alkaline solutions. The tap water at Rice Institute, which is strongly alkaline because of the presence of sodium carbonate, when used in a 1 to 1,000,000 solution of copper sulphate was apparently as effective as distilled water, even after standing for 24 hours to allow time for possible precipitation of the copper.

Since organic matter in solution rapidly precipitates copper, water containing considerable quantities of it should receive larger quantities of copper sulphate to make up for loss by precipitation. The concentration of copper sulphate necessary to destroy typhoid bacilli, according to Rettger and Endicott (*17*), was four times as great in water containing 0.01 per cent peptone as in distilled water and 40 times as great in the presence of 1 per cent peptone. Moore and Kellerman (*12*) advise an increase of 2 per cent in the concentration used to kill algae for each part per 100,000 of organic matter. It is probable that a similar increase in the amount of copper used against snails would be sufficient to counteract the effect of the organic matter.

The presence of algae in the water has a marked effect on the action of copper salts on snails, since the algae, which are killed by the salts, absorb them. Bado (*2*) has demonstrated considerable quantities of copper in the ash of algae which had been exposed to copper sulphate in dilute solution, and he states that it is absorbed at different rates by different species. In a preliminary experiment, the writer found that snails placed in one liter of a 1 to 1,000,000 solution of copper sulphate, together with a large handful of algae (Vaucheria and attached diatoms) although they showed symptoms for a few hours after immersion, subsequently revived and on the following day were as active as the controls. To test more accurately the effect of algae, a quantity of fresh green algae was rinsed and then squeezed like a sponge until water was no longer expelled by moderate pressure. Quantities of this weighing 0.25, 0.5, 1, 2, 3, 4, and 5 gm. were placed in liters of a 1 to 1,000,000 copper-sulphate solution, and snails (*Physa occidentalis*) were placed in each. The snails in the jars containing up to 1 gm. of the wet algae (1 gm. = 150 mgm. dry weight) died as quickly as did the controls in a simple copper-sulphate solution. Those in jars containing 2, 3, and 4 gm., although prostrate within 24 hours, still responded weakly to stimuli at the end of 48 hours but did not revive when placed in fresh water. One-third of the snails in the jar with 5 gm. of algae partially revived in the solution. A second experiment, similarly conducted, but with the use of Spirogyra, one of the algae most susceptible to copper salts, was tried. In this experiment only 1, 2, and 3 gm. quantities were used. The snails with 1 gm. of Spirogyra did not die within 48 hours but failed to revive in fresh water and died within 48 hours after being refreshed. Of those with 2 gm. 50 per cent revived after being refreshed, whereas of those with 3 gm. all revived.

As shown in the preliminary experiments with various chemicals, chlorinated lime up to double the amount used for sterilizing drinking water does not affect snails at all. It was found, furthermore, that the presence of chlorinated lime in the proportion of 1 to 250,000 (about 1.3 parts available chlorin per million) had an inhibiting effect on the action of copper sulphate on snails to such an extent that some specimens did not even become prostrate in the solution. The mode of interaction of the copper sulphate and chlorinated lime was not investigated, but it is possible either that the liberated oxygen from the chlorinated lime may counteract the effect of the copper sulphate on the enzyms, or that a chemical reaction takes place which precipitates the copper. If the latter is true it might be feasible to remove copper sulphate from solution in water by the use of chlorinated lime, in case this should for any reason be desirable after using it in destroying algae, snails, or other organisms.

A number of practical field experiments were carried out to demonstrate the effectiveness of copper-sulphate treatment for snails in actual practice.

The first experiment was conducted on a pool in the vicinity of Corvallis estimated to contain about 113,000 liters of water. This pool was a portion of a stream which dries up during the summer, leaving isolated bodies of water, probably connected by seepage through the sandy substratum. The pool contained patches of Spirogyra here and there together with a number of higher aquatic plants (Veronica, Cicuta, and others). The fauna included frogs, newts, and stickle-backs among vertebrates, and a great variety of insect life, the most abundant forms being Notonectids, Corisids, damsel fly larvae, neuropterous larvae, and beetles of various kinds, both adults and larvae. Five species of molluscs were present. *Physa occidentalis* and the small bivalve *Musculium walkeri* were abundant in the aquatic vegetation. *Fluminicola fusca* was abundant, and *Goniobasis plicifera* was fairly common on the sandy bottom, especially around the edges of the pool, and an unidentified Planorbis occurred sparingly in the vegetation.

On August 26, 113 gm. of commercial copper sulphate were dissolved in about 10 liters of water and sprinkled on the surface of the pool by means of a watering pot, making approximately a 1 to 1,000,000 solution, but without making any allowance for impurity of the copper sulphate, absorption of algae, combination with organic matter in solution, or dilution by seeping in of fresh water.

The effect of the experiment was studied 48 hours later. The masses of algae had been killed, but the higher plants, vertebrates, including the stickle-backs, and the various kinds of insects were apparently unharmed. No living specimens of Fluminicola or Planorbis could be found, though hundreds of dead ones were seen lying on the bottom. The majority of the Physae were dead, but a few seemed to be merely prostrate. Some specimens of Goniobasis were withdrawn into their shells and were evidently not dead. All the Musculium were lying on

the bottom with their shells tightly closed. Another examination was made on August 30, and at this time all specimens of Physa, Fluminicola, Planorbis, and Musculium and the majority of the Goniobasis were dead, but about one-third of the last had revived and were apparently well again. This fact was evidence that practically all the copper sulphate had been removed either by absorption by the algae or by the dissolved organic matter, increased by the disintegration of thousands of snails or by seepage through the sandy substratum, since it had previously been fully demonstrated that Goniobasis remained prostrate even in a 1 to 5,000,000 solution of copper sulphate.

A similar experiment was carried out on another pool of similar kind and with practically the same fauna and flora; this pool was, in fact, another isolated portion of the same stream. This time a copper-sulphate solution of 1 to 500,000 was made. All molluscs were apparently dead in 48 hours, and none subsequently revived. No other higher animals were affected at all.

To test the use of copper sulphate for destroying snails in a flowing stream, an experiment was attempted in Oak Creek, near Corvallis, Oreg. The water in this creek is cold and clear and flows rapidly. The stream is very uneven as to width, depth, and speed, consisting, in fact, of a series of sluggish pools connected by rapids and cascades. At this season of the year, September 1, the stream was very low, and was found to flow only about 550 liters per minute. The stream contained enormous numbers of *Goniobasis plicifera*, the bottom in some places being fairly covered with them.

To treat this stream a 7-gallon keg fitted with a drawn-out glass spigot which would feed a solution into the stream at an average rate of 1.5 liters per hour was filled with a copper-sulphate solution strong enough to make a 1 to 500,000 solution in the stream. This strength of solution was used to make allowance for combination with organic matter, precipitation in other ways, and error in estimation of the volume of the stream. The experiment ran smoothly for about 14 hours, and at the end of this time the snails for at least a mile down the stream were prostrate and apparently dead. Meanwhile, however, a rain storm came up which in the following 10 hours approximately tripled the volume of water in the stream. An attempt was made to strengthen the solution fed into the water at a corresponding rate, and this seemed to be successful. Pressure of other duties made it impossible to visit the experiment again until 48 hours later. At this time it was found that the spigot had been plugged by a particle of débris, though precautions had been taken to keep the solution as clear as possible. The cessation of flow had evidently occurred shortly after the experiment had last been visited, consequently the stream had been treated little more than 24 hours. A few of the snails were dead, but the majority had revived and were as active as ever.

On account of the writer's moving from Corvallis, Oreg., to Houston, Tex., a few days later, this experiment could not be repeated on Oak Creek, but a similar experimemt was made on a small stream or "bayou" a short distance from Houston. This stream, flowing about 1,500 liters per minute, is sluggish, fairly even in width and depth, and contains water moderately alkaline and rich in lime. The only abundant snail in the stream was a small Ancylus which occurs on dead leaves on the bottom. A few specimens of *Physa anatina* were obtained at each dredging.

To treat this stream a 10-gallon barrel was used, fitted with a glass spigot as before but protected from plugging up by the use of a glass funnel with the large end inside the barrel, this being covered with cheesecloth to strain the solution as it flowed out. The addition of a few cubic centimeters of sulphuric acid prevented the flocculent precipitation of iron sulphate, which is present as an impurity in commercial copper sulphate. The diminution in rate of flow from the spigot resulting from a lowering of the level of the fluid in the barrel follows a parabolic curve, in this case decreasing fairly steadily from 50 cc. to 30 cc. per minute until the barrel was half empty. To prevent a greater fall in pressure a 20-liter jar was placed above the barrel and connected with it by an automatic siphon, so that the contents of the jug would be utilized when the barrel was half empty. A simpler method would have been the utilization of a tube equal to the height of the barrel to give a greater head. By this method the entire contents of the barrel could be utilized before refilling without too great a change in the rate of flow of the copper solution. The experiment was allowed to run for 72 hours, although 48 hours' exposure to the copper solution had been found experimentally to be sufficient to kill snails. However, in a flowing stream it was thought advisable to give an extra day to make up for uneven flow and dilution in the deeper portions of the stream during the early part of the experiment and to give time for diffusion into the "dead" water along the sides of the stream. At the end of the experiment—that is, for the last 12 hours—the lower half of the barrel was allowed to run itself out, thus gradually diminishing the strength of the solution in the stream. It was thought that in this way the actual time during which the stream was treated by a full 1 to 500,000 solution would be at least 48 hours. Three days after the completion of the experiment the stream was again dredged at intervals of about one-third of a mile at the same points at which dredgings were made prior to the experiment. A few empty Physa shells were found, but no living snails of any kind were obtained at any point along the length of the stream (about 1½ miles). It was unfortunate that the stream was not longer so that the actual distance over which the treatment was effective could be determined, but since this would

obviously vary greatly in different streams, according to evenness of width and depth, strength of current, purity of water, and possibly other factors, it would be necessary in treating any stream for the destruction of snails to determine, after the experiment, the distance over which it is effective and to repeat the experiment at a point on the stream a little above where the first live snails were found.

By utilizing a 50-gallon barrel and filling it at 12-hour intervals with a 10 per cent solution, streams running as much as 3,500 gallons per minute could be treated by this method, and, of course, by the use of several such barrels, still larger streams could be treated. Repeated attempts were made to find a method by which the copper salt could be fed into a stream at a constant rate without first being put into solution. This would, of course, save much time and labor in the treatment of large streams. A method was finally worked out by which it was hoped that this could be accomplished. Cylinders of sheet metal were carefully lined with paraffine inside to prevent any chemical action with the copper sulphate. Wooden tubes could be used as well but are not so readily obtainable as are the sheet metal tubes, which, in diameters of from 2 inches up, can be obtained from any tinsmith. A copper or bronze screen is tied over the end of the tube, and the tube is filled with copper-sulphate crystals of more or less uniform size. Commercial "pea" crystals could be used, or crystals of desired size can be obtained by sifting through two screens. The screened end of the tube is immersed about 1 cm. in the stream to be treated, and the copper sulphate is dissolved out from the bottom of the tube, a fresh supply being constantly furnished by gravity in the tube. Theoretically the copper salt should go into solution at a fairly constant rate, determined by the area exposed to the water, the speed of the stream, and the temperature of the water. Up to the present, however, it has not been found possible to make this simple apparatus work satisfactorily in practice, because of the fact that all the water in the vicinity of Houston is strongly alkaline. The alkalinity precipitates the iron sulphate contained as an impurity in commercial copper sulphate and also forms, in the course of two or three hours, considerable deposits of copper carbonates. These two substances together tend to clog the screen through which the copper sulphate is taken into solution, thus causing a rapid diminution in the rate of solution. If this difficulty could be overcome by some feasible method of keeping the water at the mouth of the tube slightly acidified, or if the water to be treated were not alkaline, large streams could be treated with comparatively little trouble by this method, using several tubes of suitable diameter at intervals across the streams. It would, of course, be preferable to treat streams at a comparatively shallow, rapid-flowing point, since this would facilitate a rapid diffusion throughout the water.

SUMMARY AND CONCLUSIONS

(1) Fluke diseases of both man and domestic animals are of great importance in many parts of the world. They are debilitating diseases of long duration and difficult to treat or cure. Preventive measures, therefore, are of great importance. The working out of preventive measures based on scientific facts has only recently become possible, since the life histories and modes of infection of the human flukes have been discovered only in the last three or four years.

(2) In all known cases fresh water snails act as intermediate hosts for the important flukes of man and domestic animals. A practical and efficient method of destroying these snails would make the ultimate eradication of fluke diseases, in spite of the difficulty in treating them, a matter of brighter prospect than the eradication of hookworm and other intestinal parasites, in which the sanitary disposal of feces must be relied upon.

(3) Experiments by the writer, carried out to find some cheap, harmless method of treating water to destroy snails, demonstrated that copper salts exert a powerful toxic effect upon snails even in very high dilution. In an experiment upon eight species of six families it was demonstrated that copper sulphate in proportions of 1 part to from 500,000 to 2,000,000 parts of water destroys snails of all these species within 48 hours; 50 per cent or more are destroyed in dilutions up to 1 to 5,000,000. From the point of view of expense, harmlessness, and convenience in use copper sulphate is preferable to any other substance which has been tried or suggested for destroying snails. The eggs of the snails are not destroyed by the copper salts.

(4) Copper salts are also highly toxic to algae, fungi, and other lower organisms but are apparently harmless, in the dilutions used, to higher plants and animals, except fish. Water treated with copper sulphate, therefore, is uninjured for drinking, bathing, or irrigation purposes.

(5) The effectiveness of copper sulphate in water is modified more or less by temperature, alkalinity, dissolved organic matter, and living algae. Some allowance should be made for these factors in estimating the amount of copper to be used in any given body of water. The proportion should vary from 1 to 1,000,000 in relatively pure water at 20° C. or above to 1 to 500,000 in water which is very cold, is alkaline, contains dissolved organic matter, or harbors an abundance of algae. If the growth of algae is very luxuriant, it would probably be advisable to kill these algae by a preliminary treatment with a 1 to 1,000,000 solution of copper sulphate, following this in the course of a few days or a week by a second treatment.

(6) Copper sulphate can be administered to ponds, reservoirs, or other bodies of standing water in the way advised by Moore and Kellerman for the destruction of algae in water. This method provides for the

solution of the correct amount of the salt from a sack attached to the back of a canoe or boat, or, in very small pools, to the end of a pole. Dissolved copper sulphate can conveniently be sprayed on small pools from a spray pump or even an ordinary garden watering pot. In most cases Bullinus, Physopsis, Planorbis, and Limnaea could be destroyed by these methods.

(7) For the treatment of running streams the use of a barrel of suitable size, fitted with a screened spigot, is recommended. The barrel is filled with water, and sufficient copper sulphate is dissolved into it so that the desired amount will be fed into the water per hour. Inasmuch as no two spigots will flow at exactly the same rate and since the rate of flow will diminish as the level of the fluid in the barrel is lowered, it is necessary to determine beforehand the rate of flow at different levels and to calculate the amount of copper sulphate to be dissolved according to the average rate of flow. By the use of a tube of equal or greater length than the height of the barrel, so that the head is increased, the diminution in rate of flow can be greatly lessened. The addition of a few cubic centimeters of sulphuric acid to the solution in the barrel prevents the precipitation of iron sulphate, which is present as an impurity in commercial copper sulphate and tends to clog the filter. Melania and Blanfordia would probably have to be attacked by this method, since they live in flowing water.

(8) In water which is not alkaline, large streams could be treated more easily by allowing the copper sulphate, in the form of uniform crystals, to dissolve directly into the stream through the screened end of a tube. The amount of salt which would go into solution per unit of time would depend on the diameter of the tube, the speed of the stream, and the temperature of the water. If some feasible method could be devised for slightly acidifying the water at the point where solution of the salt is taking place, this method could be used advantageously in all but very small streams.

(9) It is believed that by attacking the intermediate hosts of the various pathogenic flukes of man and domestic animals by the use of copper sulphate as herein outlined trematode diseases can successfully be brought under control and can either be greatly reduced or entirely eliminated in endemic areas, and this with comparatively little expense and without active cooperation on the part of natives. With Government aid and supervision, the work being carried out under the direction of scientifically trained men or commissions, it seems entirely possible that entire States or countries, at least in the vicinity of towns and villages, could be freed of human fluke diseases, and that seriously affected districts where sheep and cattle are raised could have the fluke scourge wiped out in a short time with little expense.

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INJURY TO SEED WHEAT RESULTING FROM DRYING AFTER DISINFECTION WITH FORMALDEHYDE

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INTRODUCTION

Much has been written on the use of formaldehyde as a fungicide for wheat and other grains infested with smut, but relatively little has been carefully done on the effect of such treatment on the seed. The usual recommendation has been a dip of about 10 minutes in a solution consisting of 1 part of commercial formaldehyde solution to 320 parts of water, followed by a 10-minute drain. Almost without exception instructions are given to dry the seed thoroughly before storing it. The frequent advice that it be sown immediately after treatment and not stored indicates that it has been learned by experience that injury to the grain occurs not so much from the treatment as from holding it in storage afterward. However, it has been almost universally concluded, without experimental evidence, that damp storage causes the injury. Thus, practically every publication dealing with seed treatment carefully warns against the storage of formaldehyde-treated seed that has not been thoroughly dried after treatment.

The present investigation of the post-treatment action of formaldehyde on seeds was begun in 1918 in the plant pathology laboratories of the University of California as a part of the cereal-smut eradication campaign carried on by the United States Department of Agriculture and was continued through a period of nine months. The major conclusion reached is that it is extremely hazardous to dry seed which has been treated with formaldehyde solution,[2] and that, contrary to common belief, seed wheat is absolutely uninjured by a 0.1 per cent solution (1 to 40) and, if kept moist, may be held indefinitely without injury, unless attacked by molds. We believe that the data here presented will contribute to our knowledge of the physical and chemical properties of formaldehyde and the relation of these properties to physiological processes in the seed. Such knowledge will undoubtedly

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[2] Reports sent in to Dr. H. B. Humphrey and to Prof. W. W. Mackie of occasional poor stands of wheat from treated seed sown by farmers in the dry regions of California and Oregon indicate that field results confirm those arrived at through these experiments.

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be helpful in any consideration of the more practical problems connected with the use of this chemical as a fungicide.

Certain investigators working on this problem have shown that injury to formaldehyde-treated seed occurs when the seed is allowed to dry after treatment. The earliest report we have found of such work is that of McAlpine (*11*),[1] whose experiments showed that seed treated with a solution of 1 pound of formaldehyde in 40 gallons of water just prior to sowing under conditions favoring immediate germination grew as well as untreated seed. If, however, the seed was allowed to dry for a day or more before germinating or if it remained in dry soil some days before a rain, it suffered extreme injury. He gives instances of such injury reported by farmers who from experience had learned to sow formaldehyde-treated seed in moist soil immediately after treating. McAlpine attributed this injury to the hardening effect of formaldehyde on the seed coat. He claimed that by soaking the dried treated seed in water prior to sowing this injury was averted. He further stated that the injury after a dip in a 1 to 40 solution was most pronounced when the seed had been kept a week after treatment. After two weeks it began to improve until, when sown a month after treatment, it was practically as good as 24 hours after treatment. He stated also that this recovery did not occur when the solution used was twice as concentrated.

In 1908, Shutt (*14*) found that a delay of three days in sowing after the formaldehyde treatment reduced the percentage of germination and increased the proportion of weak and slender plants. In opposition to this are the results reported by Hurst (*8*), who states that seed may be—

> treated and kept for any reasonable length of time without affecting its vitality.

Some of his samples, he says, had been treated 12 months before and germinated as well as the untreated seed. Stewart and Stephens (*16*) found that after the use of a 1 to 50 solution their samples were uninjured by 6 weeks' dry storage, which was the longest storage period tested. Brittlebank (*3*) noted a falling off in the germination of seed treated with formaldehyde solution after being kept dry a week, the decrease continuing to the sixth week, after which the percentages rose and fell with various fluctuations through the remainder of the 54 weeks. Güssow (*6, p. 21–22*) reported some figures obtained by Dr. C. E. Saunders, Dominion Cerealist, showing that treated seed which originally germinated 75 per cent was entirely killed after being stored dry a year. Some barley and oats treated similarly were almost wholly killed after standing dry a year.

The first investigators to connect this storage injury with that property of formaldehyde by virtue of which it forms a solid condensation product or polymer upon evaporation were Darnell-Smith and Carne (*5*), who

[1] Reference is made by number (italic) to "Literature cited," p. 243–244.

attributed the conflicting reports of the injury resulting from the formaldehyde treatment to variations in the deposit of this polymer on the seed as it dried. They found low germination percentages and defective seedlings to result from the drying of treated seed. Their results do not agree with those of McAlpine, which were responsible for the latter's conclusion that soaking in water prior to sowing removed the cause of injury. They did find, however, that washing immediately after treatment prevented subsequent injury in storage by removing the source of the deposit. They thought that there was no internal poisoning of the seed before germinating but that there was some deleterious chemical action of a formaldehyde salt in the pericarp, which was alleviated by soaking. Müller and Moltz (*12*) proved that the polymer, paraformaldehyde, when mixed with the soil was very injurious to wheat sown in it.

An interesting and comprehensive report on the secondary effects of formaldehyde treatment is the recent article by Kiessling (*9*). He obtained severe injury upon storing treated seed which had been dried, and this injury he found to be cumulative as the duration of the storage period continued. He also was unable to confirm McAlpine's statement that soaking the dried seed before sowing prevented the injury. Although giving adequate and convincing proof that dry storage is more fatal than damp storage, he does not advance any explanation.

Coons (*4*) also found that it is unwise to hold formaldehyde-treated grains any length of time and that the injurious action is cumulative when the treating solution is dried on the seed. He suggests that this injury may be due to the formation of the solid condensation product, paraformaldehyde, which might persist on the grain even after months of drying.

POST-TREATMENT ACTION OF FORMALDEHYDE ON DRYING SEED WHEAT

Except the studies of Coons (*4*) and those of Stewart and Stephens (*16*), it will be noted that all the work on dry-storage injury to wheat has been done outside the United States. This no doubt accounts for the fact that it has been generally overlooked in this country or at least has not resulted in any modification of the widespread instructions relative to drying formaldehyde-treated wheat before storage. It was to investigate this supposed formaldehyde injury to damp stored seed that the studies here recorded were begun. These experiments resulted in the rediscovery of the fact that so long as the seed treated with a 0.1 per cent (1 to 40) solution remains damp there is no injury from the chemical but, when dried, the seed is variously injured, depending upon the manner of drying and upon the moisture content of the atmosphere surrounding the seeds.

In the following experiments the seeds were left for 10 minutes in a 0.1 per cent solution of formaldehyde followed by a draining period of 10 minutes. This strength is equivalent to 1 pint in 40 gallons of solution, varying in small degree from that commonly referred to as 1 to 40, which means 1 pint of standard formaldehyde solution in 40 gallons of water. As the formaldehyde solution used in the laboratory contained 36.2 per cent formaldehyde, such a dilution would be 1 part of formaldehyde in 884 parts of solution, or 0.113 per cent. Unless otherwise stated, the wheat used was Little Club with a low percentage of thrashing injury. After treatment the seed was spread on towels for an hour in order to remove excess surface moisture. The damp seed was then divided into two lots. One lot was put into three Mason fruit jars, holding about a quart each, and sealed. The other lot was put into three boxes, 4 by 5 by 6 inches, and left uncovered. They were stirred frequently throughout the experiment. These boxes each contained the same quantity of wheat as did the jars. The original idea in having three samples of each seed lot was to determine the relation of temperature to the injury which was expected to appear in the damp samples. One box and one sealed jar were left in the refrigerator at a temperature of 10° C., one of each in the laboratory at 20°, and one in the greenhouse, where the temperature averaged about 30°. For each of the six samples, as in all subsequent experiments, there was a control of seed dipped in water instead of formaldehyde.

The following germination tests were made on blotters placed in square pans, 12 by 12 inches, 1¼ inches deep, kept at room temperature. The pans were covered with square pieces of glass, which made it easy to observe the progress of the germinations. The depth of the pans gave the seedlings a chance to grow erect and more normally than would be the case if they were grown between blotters. Only those seeds were called germinated which produced both a root and plumule. Many which did so were too severely injured to produce plants in soil, but the approximate percentage of these was obtained by contemporaneous soil germinations (Table II). Soil germinations have the advantage of approximating more closely field results. The many advantages in the use of blotters, however, lead the writer to emphasize the fact that they are just as valuable to show the occurrence and comparative degrees of seed injury. In view of the possibility of earlier detection and easier study of such injury, they even may be preferable. The results of the blotter germination tests are given in Table I.

TABLE I.—*Percentage of germination of wheat treated with 0.1 per cent formaldehyde solution and variously stored*

Treatment and storage.	Stored 0 days.	Stored 1 day.	Stored 3 days.	Stored 6 days.	Stored 10 days.	Stored 14 days.	Stored 21 days.	Stored 28 days.	Stored 35 days.	Stored 42 days.	Stored 60 days.	Stored 120 days.
Stored in refrigerator at 10° C.:												
Treated, stored dry	100	100	98	[a] 90	[a] 100	[a] 92	[a] 92	[a] 74	[a] 74	[a] 72	[a] 52	[a] 10
Control, stored dry	99	100	100	98	100	98	98	98	98	100	100	98
Treated, stored damp	100	98	92	90	98	92	100	94	98	100	[b]	[b]
Control, stored damp	99	98	98	100	100	98	100	100	96	[b]	[b]	[b]
Stored in laboratory at 20° C.:												
Treated, stored dry	100	100	[a] 92	[a] 92	[a] 84	[a] 72	[a] 76	[a] 86	[a] 72	[a] 64	[a] 60	[a] 32
Control, stored dry	98	98	98	100	98	100	100	98	100	98	100	98
Treated, stored damp	100	98	98	98	100	98	98	[b]	[b]	[b]	[b]	[b]
Control, stored damp	99	100	98	100	98	[b]	[b]	[b]	[b]	[b]	[b]	[b]
Stored in greenhouse at 12° to 35° C.:												
Treated, stored dry	100	98	[a] 90	[a] 90	[a] 84	[a] 96	[a] 90	[a] 90	[a] 96	[a] 98	[a] 90	[a] 82
Control, stored dry	99	100	94	100	98	94	100	100	96	98	100	98
Treated, stored damp	100	96	94	98	100	94	100	100	100	98	100	94
Control, stored damp	99	100	100	100	100	92	96	[b]	[b]	[b]	[b]	[b]

[a] These seedlings were markedly retarded and injured, as indicated by slow germination and deformed plumules. The lower the percentage of germination the more extreme is this malformation of those which succeed in producing the plumule and root. Therefore, the percentage figures for the dried, treated seeds, especially those from the greenhouse, do not indicate the real extent of their injury.

[b] Attacked by molds.

The outstanding fact shown by these experiments is that all the seed which was treated with the formaldehyde solution and then dried by being allowed to stand open to the air was either killed or seriously injured after three to six days, while that treated at the same time and stored at the same temperatures, but kept damp by being sealed in jars, was practically uninjured up to the time it was destroyed by molds. Later experiments have shown that injury may appear in dry-stored seeds in less than three days, depending on the manner of drying. The dry controls maintained the original germination throughout, and the wet ones did also until they were killed by the development of fungi in the jars. It will be noticed that molds appeared more slowly in the damp, treated seed than in the damp controls, giving evidence of the fungicidal action of the formaldehyde remaining on the seed. The reason for the more extreme injury in the lots stored at room temperature and in the refrigerator compared with those in the greenhouse will be discussed later. These percentages also show most strikingly that the injury to dried seeds is cumulative and that there is no recovery. This is borne out by all subsequent experiments and refutes the claim of McAlpine (*11*) and Darnell-Smith and Carne (*5*) that there is a steady improvement after the extreme injury which appears after a week or so.

In addition to low germination percentages, the injured samples showed a characteristic deformity and extreme retardation of the injured seedlings. The earliest appearance of injury in the dried seeds was simply a noticeable retardation of germination in the samples after being stored three and six days, the plumules and roots never catching up with those of the uninjured seedlings. The retardation became more extreme as storage continued, with an ever-increasing number of short plants which grew very slowly and resulted in stunted and misshapen plumules and underdeveloped roots. After 10 days' storage all the seeds of the three treated and dried lots were thus inhibited, so that upon germinating they presented the appearance shown by those in Plate 36, A. The characteristic deformity by which this extreme formaldehyde injury can always be detected is the curving of the plumule as it emerges until it is sickle-shaped (Pl. 36, B). The growth of the sheath is inhibited so that it never grows more than a few millimeters, leaving the young leaves to push out unprotected, spindling, and weak, unable to push their way through soil. The roots are underdeveloped but show no deformity. It has been noted throughout these experiments that the greater the retardation of germination in any injured seed lot, the greater the proportion of weak, spindling plants produced. Whether the effect of the formaldehyde on the sheath is to stop growth by stopping cell division or by inhibiting the growth of the cells after they have divided was not determined.

Anyone observing the seeds of the injured dry lots, the uninjured damp ones, and the controls, germinating in blotters where invasion by Rhizopus

was possible, would notice at once the luxuriant growth of mycelium on the injured seeds and its comparative rarity on the uninjured ones. He might be inclined to ask whether the injury of the former samples was not the result of fungous activities instead of action of formaldehyde which might by its presence simply stimulate the growth of the mold. This question is easily answered by disinfecting some of the dried, treated seeds by a 10-minute dip into a 1 to 1,000 solution of mercuric chlorid and germinating them on sterile blotters. The seedlings show the same characteristic injury, but the percentages of germination are higher, though not normal. This is because when they escape infection some of the injured seeds succeed in germinating and produce weak plants. These seeds, had they not been disinfected, would have been killed by the invading fungus before the retarded root and plumule could emerge. The extent of the development of this fungus on the various lots of germinating seeds serves as a fairly accurate index of the injury done to the seed by the treatment. It is concluded from such experiments and many others showing the same fact, which will be reported in detail in a subsequent paper, that injury from drying after the formaldehyde treatment predisposes the seed to attack by molds, especially Rhizopus, the chemically injured embryo being unable to resist infection.

It is commonly believed that blotter germinations are worthless so far as being an indication of the viability of seeds in soil. Therefore, along with the blotter germinations summarized in Table I, occasional tests of the stored seeds were made in pots of sandy loam soil in the greenhouse. It was found that with the uninjured samples the soil germinations gave the same results as those made at the same time in blotters. With injured seeds they were lower, as was to be expected, for in the blotters all those seeds were counted germinated which produced both root and plumule even though these were stunted or deformed. In the soil such seedlings would never reach the surface, and so the count of germinated plants from injured seed lots would be lower. Consequently, the injury produced by drying the formaldehyde treated seeds appeared even more strikingly in the soil and would more closely approximate actual field results. This is shown in Table II.

These figures do not indicate the full extent of the injury suffered by the dried treated seed. Many of the seedlings from the injured samples are short and spindling, while none of this sort are found in the controls or in the samples which had been stored damp (Pl. 37, A). This same extreme injury was shown by the seeds stored dry in the laboratory, but the figures are not included in Table II, because the damp controls of both the untreated and the treated seed were destroyed very quickly by the rapid development of Penicillium and Aspergillus at that temperature. Plate 37, B, shows the seedlings produced by these three seed lots injured by drying and the seedlings produced by two of the controls.

TABLE II.—*Percentage of germination in potted soil of wheat treated with 0.1 per cent formaldehyde and stored for various periods*

Treatment and storage.	Stored 10 days.	Stored 32 days.	Stored 56 days.
Stored in refrigerator at 10° C.:			
Treated, stored dry	62	28	18
Control, stored dry	100	100	100
Treated, stored damp	100	[a] 92	[a] 90
Control, stored damp	100	[a] 92	[a] 76
Stored in greenhouse at 15° to 35° C.:			
Treated, stored dry	60	54	74
Control, stored dry	98	100	98
Treated, stored damp	96	96	100
Control, stored damp	96	[a] 94	[a] 82

[a] The germination of these samples is lowered by the development of molds in the jars. As will be reported in a subsequent paper, saprophytic fungi attack stored wheat whenever the humidity is 70 per cent or more, the treated seeds being attacked more slowly because of the slight protection afforded by the formaldehyde.

After it had been determined that wheat stored and allowed to dry after treatment was seriously injured, the next question which arose was whether the same injury would be produced if seed sown immediately after treatment in dry soil remained there for some time before sufficient rain fell to dampen the soil and induce germination. In dry regions wheat often lies in the soil for weeks before germinating. To duplicate these conditions, seed was treated in the usual manner with a 0.1 per cent solution of formaldehyde and sown, 50 seeds in a pot, in air-dry soil. On one series, a 0.2 per cent solution was used to show more strikingly the cumulative nature of the injury. One pot of each, with a control of seed treated similarly with water, was watered after predetermined intervals such that the first pot was watered and started to germinate immediately after planting while the last one remained dry for a month. The results of the experiments with wheat are given in Tables III and IV.

TABLE III.—*Percentage of germination of Little Club wheat after lying in dry soil (Yolo clay loam) following treatment with 0.1 per cent formaldehyde solution*

Treatment.	Water applied after—								
	0 days.	1 day.	2 days.	3 days.	4 days.	5 days.	7 days.	10 days.	14 days.
Treated	98	94	94	86	94	64	52	52	42
Controls, soaked in water	100	98	100	98	98	98	98	98	100

The data in Table III indicate that it is not safe to treat wheat with formaldehyde, even when the strength of solution is as weak as 0.1 per cent, if the seed must be sown in very dry soil without certainty of rain within a few days.[1] Besides a lower percentage of germination, the ger-

[1] Field reports are found to be in agreement with these laboratory tests. The hitherto unexplainable poor stands of wheat from treated seed obtained by the farmers of the dry regions of California can now be safely attributed to the fact that the seed lay in the dry soil for some time before rain.

mination of the injured seed lots was retarded, often several days, and they produced a considerable number of spindling or short plants which apparently never would be strong (Pl. 38, A).

The injury from drying, either in storage or in the soil, is greater the more concentrated the solution used. The data given in Table IV demonstrate this fact, the experiment differing from that summarized in Table III only in the use of sandy-loam soil instead of the heavy Yolo clay loam and in the fact that a parallel experiment was run at the same time in which some of the treated seed was kept in a box in the laboratory and a sample was germinated in blotters after drying for periods corresponding to those in the soil experiment (Pl. 38, A).

TABLE IV.—*Percentage of germination of Little Club wheat treated with formaldehyde and dried, both in the soil and in the air*

Length of drying period.	Sown in dry soil.			Dried in the air and germinated in blotters.		
	0.1 per cent formaldehyde solution.	0.2 per cent formaldehyde solution.	Control, dipped in water.	0.1 per cent formaldehyde solution.	0.2 per cent formaldehyde solution.	Control, dipped in water.
Days.						
0	100	92	98	98	98	96
2	84	68	.....	98	98	100
5	84	66	.....	90	60	100
7	80	60	.....	84	66	100
10	86	48	.....	86	50	96
14	74	34	.....	90	52	96
20	88	44	.....	62	54	96
30	62	48	100	80	44	96

In none of the experiments summarized in Tables I to IV was there any injury to seed germinated at once after the dip into either 0.1 per cent or 0.2 per cent formaldehyde. This fact is not in agreement with results reported by many experimenters. Stewart and Stephens (*16*), for instance, found that an immersion of 10 minutes in a 1 to 40 solution (0.1 per cent) caused almost a 50 per cent loss. Kiessling (*9*), for example, notes the great variation in the results reported on the effect of formaldehyde on germination of seed. He concludes from the work of others and from his own experiments that formaldehyde produces a serious effect on the seed, the degree of injury depending on the sensitiveness of the different varieties and the condition of the sample. None of the wheat varieties tested in this laboratory (Little Club, Early Baart, Marquis, Defiance, Sonora, and White Australian) was ever found to be injured in the least by the recommended treatment, or by one twice as strong, whether germinated in blotters or in the soil, so long as it was sown immediately after treatment. Not only will the seed be uninjured by the usual 20-minute exposure to a 0.1 per cent solution but it will

stand an immersion of 8 hours without injury. It can remain 1 hour without injury in a solution twice as strong. Table V shows the result of an experiment to determine the resistance of Little Club wheat to long exposures to various strengths of formaldehyde solutions.

TABLE V.—*Relation between strength of solution, duration of exposure, and seed injury*

Strength of solution.	Soaked 20 minutes.		Soaked 1 hour.		Soaked 6 hours.		Soaked 8 hours.		Soaked 24 hours.	
	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.
	Per ct.	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*
Saturated	0	.....	0	.....	0	.....	0	.....	0	.....
4.50 per cent	100	3.5	25	0.2	0	.....	0	.....	0	.....
0.45 per cent	95	3.5	90	.6	0	.....	0	.....	0	.....
0.20 per cent	100	3.5	95	3.5	40	1.0—	15	1.0—	0	.....
0.10 per cent	100	3.5	95	3.5	100	3.5	95	3.5	85	2.5
Control, untreated	100	3.5	95	3.5	100	3.5	95	3.5	95	3.5

Table V shows that Little Club wheat, thrashed with little injury, will stand an 8-hour exposure to a 0.1 per cent solution, a 1-hour exposure to a 0.2 per cent solution, or a 20-minute exposure to 0.45 per cent and 4.5 per cent solutions.

The post-treatment injury from dry storage after subjection to a 0.1 per cent solution as well as to stronger ones has been demonstrated not only with Little Club and Early Baart wheat but with Sonora, Marquis, Defiance, and White Australian.

PHYSICAL PROPERTIES OF FORMALDEHYDE AND PARAFORMALDEHYDE

After the fact had been established that a 0.1 per cent solution is innocuous but that the drying of this solution on the seed is harmful, the next step was to investigate the physical and chemical properties of formaldehyde in order to find a cause for the injury and a means of avoiding it. The natural supposition was that the injury is due either to a concentration of the solution on the seeds as they dry or to a coating of paraformalmaldehyde left upon them as the solution evaporates. It seemed at first inexplicable, however, that the seeds stored damp, or even wet, should remain absolutely uninjured indefinitely. In an effort to connect these facts with the possible persistence and disappearance of the chemical on the seed some qualitative tests for formaldehyde in washings of the damp and dried seed were undertaken. It was the result of these first qualitative tests which led to the intensive study of the behavior of formaldehyde solution and paraformaldehyde and the possible determination of the cause of seed injury reported in this paper.

To detect the presence of formaldeyde on treated seed, Tollen's "silver mirror" aldehyde test [1] was used. To obtain comparable water extracts of the seed lots a uniform procedure was adopted which consisted in extracting 15 cc. of the wheat sample with 10 cc. of distilled water for two minutes in a 100-cc. graduated cylinder which was rotated and shaken constantly to wash all the seeds as thoroughly as possible. Five cc. of the washings were then transferred to a test tube by means of a pipette. Extracts of all the wheat samples to be studied were thus prepared before proceeding. This is because it was necessary to add the reagent to all at as nearly the same instant as possible in order that results given by color changes might be comparable, since it is by the relative rapidity of their appearance that the relative quantities of precipitate formed by the presence of formaldehyde are shown. One cc. of Tollen's reagent was then added quickly to each tube by means of a pipette, and the tubes were watched for the appearance of the black, or, at first, dark brown precipitate indicating the presence of formaldehyde. The relative quantities of formaldehyde present in the tubes were shown by the rapidity of formation and by the density of this precipitate.

Several interesting facts were disclosed by the application of this test to the washings of treated seed. In the first place, distinct and positive reactions were invariably obtained from seed which had been drying for weeks, thus giving a clue to the reasons for the cumulative injury suffered by seeds in drying. Positive reactions were given by extracts of samples, the germinations of which were reported in Tables I and II, after the seed had dried nine weeks in the laboratory. This, however, was longer than the average persistence of the paraformaldehyde, which, on account of its volatility, usually disappeared in a month, depending on the conditions of drying. It is understood, of course, that, in the presence of moisture, paraformaldehyde at once breaks down and is again formaldehyde in solution.

In addition to this proof of the persistence of formaldehyde on the seed in the form of paraformaldehyde, the qualitative tests showed invariably that about 24 hours after treatment there was more formaldehyde on the seed stored damp in a sealed jar than on that treated at the same time and stored dry, showing a diminution in the quantity as the seed dried. After 48 to 72 hours, the amount on the seeds in the sealed jars had diminished at a more rapid rate, so that extracts from them gave weaker and slower reactions than those from the dried seed. Within a

[1] Tollen's reagent is an ammoniacal solution of silver nitrate which when added to a dilute aldehyde solution produces a black precipitate or, upon standing and in the presence of a sufficient amount of the aldehyde, forms a silver mirror by the precipitation of metallic silver on the sides of the test tube or other container. It is made by dissolving 3 gm. of silver nitrate in 30 gm. of water and 3 gm. of sodium hydroxid in 30 gm. of water, the two solutions being kept separate until ready for use, when they are mixed in equal parts by volume and the resulting precipitate of silver oxid is dissolved by the addition, drop by drop, of ammonia (specific gravity 0.923).

week, or at most two weeks, the damp seed ceased entirely to give any formaldehyde reaction. An odd reddish brown color resulted when Tollen's reagent was added to these extracts, but there was no black precipitate. The question was to determine where the formaldehyde had gone, for it seemed extremely inconsistent that it should disappear in a sealed jar and yet remain on seed open to the air. The answer was suggested by Dr. C. W. Porter, organic chemist at the University of California, who said that it probably was absorbed by bacteria and mold growing in the damp wheat.

To determine whether this were the case, some treated seeds were divided into several lots. Part were inoculated with the spores of Penicillium and sealed in small jars. The rest were left uninoculated and stored similarly. Within a few days extracts of the former samples ceased giving the formaldehyde reaction and produced the peculiar reddish brown color noted above. The uninoculated lots continued to show the presence of the chemical for some days longer but eventually became moldy and then gave the same reddish brown color with the ammoniacal silver nitrate.

Having demonstrated the persistence of formaldehyde on drying seed and its disappearance from seed stored damp, and having evidence pointing to the fact that seed injury from this fungicide may be dependent on the formation of paraformaldehyde on the seed, we next undertook a more critical study of the evaporation and polymerization of formaldehyde solutions.

It was found, upon evaporating the undiluted commercial solutions, that a surprisingly large quantity of the solid, white, condensation product was produced from comparatively small volumes. The percentage by weight of the solid formed varied greatly in different determinations because of variations in the conditions affecting the rate of evaporation—namely, quantity of solution, area of free surface, atmospheric humidity, temperature, etc. Even with these factors controlled, the same percentage could not be obtained with successive determinations because there is continuous evaporation of the solid paraformaldehyde after it has formed, as well as of the moisture in the, at first, waxy residue. In our determinations a procedure as nearly uniform as possible was always followed—that is, 50 cc. of undiluted 36.2 per cent formaldehyde solution were evaporated by exposure to the air in a 100-cc. evaporating dish, the residue being allowed to dry until the yellow color and waxy texture had disappeared. The dry residue was weighed as soon as possible after it became pure white, brittle, and easily powdered. A solution analyzed at the Insecticide Laboratory of the University of California and found to contain 36.2 per cent formaldehyde (specific gravity 1.090) produced under these conditions an average of 9.85 gm. of paraformaldehyde per 50 cc. This is 18.07 per cent of the weight of

the solution $\left(\frac{9.85}{50 \times 1.090} = 18.07\right)$ and 49.92 per cent of the weight of the formaldehyde present $\left(\frac{9.85}{0.362 \times (50 \times 1.09)} = 49.92\right)$. A 20-cc. volume of undiluted formaldehyde solution gave 16.1 per cent paraformaldehyde by weight of the solution and 44.6 per cent by weight of formaldehyde originally present in it. A 10-cc. volume, evaporated under the same conditions as the other two, gave only 7.8 per cent by weight of the solution and 21.5 per cent by weight of formaldehyde. From this and other data we know that the quantity of paraformaldehyde appearing as residue upon the evaporation of a formaldehyde solution depends on the original volume evaporated. Rate of evaporation is probably the determining factor, the extent of the evaporating surface being small in proportion to the volume as the latter is increased.

It has been shown (*10*, *14*) that dilute formaldehyde solutions grow stronger as evaporation proceeds. Notwithstanding this fact, published statements to the contrary occur in literature relating to the use of formaldehyde as a fungicide. The weakest solution analyzed by the writer was a 0.113 per cent dilution. It was found by quantitative analyses[1] of solutions before and after evaporation that the amount of formaldehyde per cubic centimeter of solution steadily increased as evaporation proceeded. Some was lost with the water, as, otherwise, the amount in the last 5 cc. would have been considerably larger than it was. The increased concentration was great enough to indicate a deposit of paraformaldehyde upon complete drying. As shown by the following test, this proved to be the case. A 0.1 per cent solution of formaldehyde was made with distilled water, and 50 cc. were put in each of two 8-cm. evaporating dishes and evaporated by leaving them exposed to the air of the laboratory, together with two controls containing 50 cc. each of distilled water. As soon as the dishes were absolutely dry (in 12 days) each dish was rinsed with 5 cc. of hot distilled water, and the washings were poured into test tubes. To each was added 1 cc. of Tollen's reagent. Results were distinct and decisive, a dark brown color appearing in the

[1] The most accurate and convenient method found for determining quantitatively the amount of formaldehyde in a solution is that of Romijn (*13*). To 5 cc. of the fomraldehyde solution are added 5 cc. *N*/10 iodin solution and so much strong sodium hydroxid solution, drop by drop, that the liquid assumes a light yellow color. After a period of 10 minutes the solution is acidified with hydrochloric acid and the free iodin is titrated back with *N*/10 sodium thiosulphate solution. Every cubic centimeter of the iodin which has been used up in the reaction with formaldehyde (the difference between the original 5 cc. added and the amount left to react with the sodium thiosulphate) represents 0.001501 gm. of formaldehyde present in the solution.

The analyses, repeated several times with approximately the same results, were obtained by evaporating 100 cc. of a 0.1 per cent solution at room temperature in an 8-cm evaporating dish. The quantity of solution used, atmospheric humidity, and other factors determine the degree of concentration of the evaporating solution at any point in the process. In the first analysis the amount of formaldehyde per cubic centimeter of solution increased from 0.0055 gm. to 0.0069 gm. after the solution had evaporated from an original volume of 100 cc. to 6 cc. (in 8 days). In a second analysis the increase was from 0.0058 gm. to 0.0069 gm. per cubic centimeter, the evaporating solution decreasing in volume from 100 cc. to 10 cc. in an equal length of time.

washings of the formaldehyde dishes, while the controls remained colorless. This showed that paraformaldehyde is left as a residue on the evaporation of solutions as weak as 0.1 per cent.

By successive weighings of the same sample it was found that paraformaldehyde is volatile, gradually breaking down and escaping as formaldehyde gas. To this property we may safely look for a large part of the seed injury following treatment with formaldehyde. Figure 1 illustrates graphically the rate of decreasing weight of 10.54 gm. of paraformaldehyde exposed to the air of the room in an 8-cm. evaporating dish in which it was originally formed by the evaporation of 50 cc. of a 36.2 per cent solution.

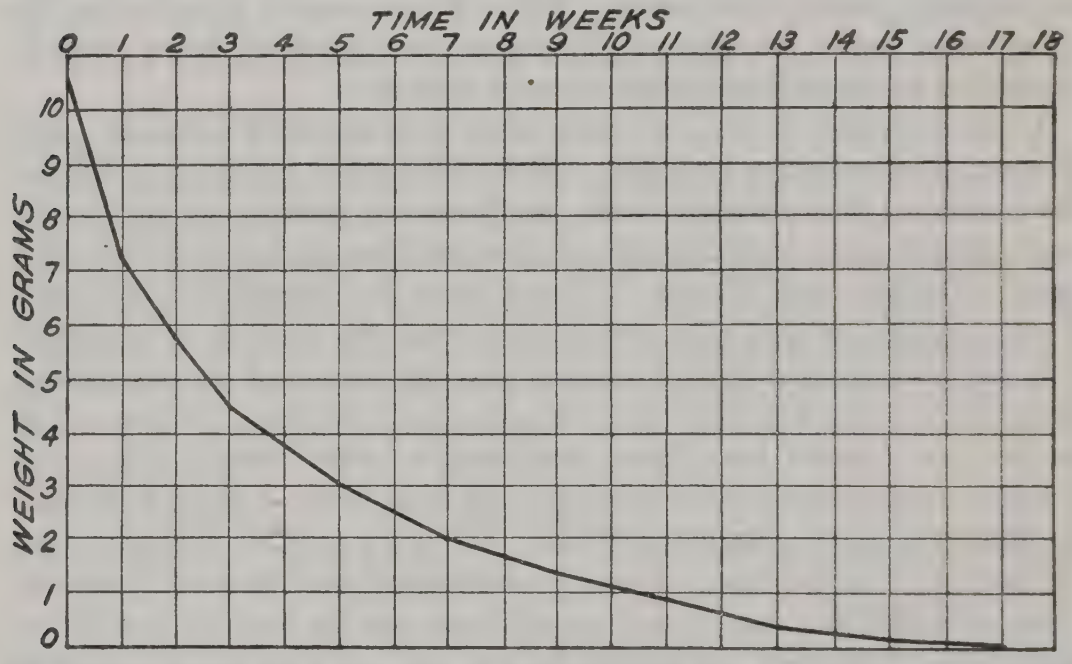


FIG. 1.—Graph showing rate of evaporation of paraformaldehyde at room temperature, approximately 20° C.

INJURIOUS EFFECT OF PARAFORMALDEHYDE ON SEEDS

After it had been demonstrated that a solid residue is left upon the evaporation of a formaldehyde solution and that this substance is constantly breaking down to form formaldehyde gas, it seemed probable that the cause of injury to treated seeds upon drying was the production of an atmosphere of concentrated gas adjacent to the seed as a result of the constant evaporation of this coating of paraformaldehyde. The gas, being heavier than air, would tend to remain around the seeds, especially when they are dried in heaps so that diffusion is not rapid. This idea was borne out by the results of an experiment showing the deleterious effect on the seed of contact with the dry, powdered, paraformaldehyde. Dry, untreated seeds were put in Syracuse watch crystals and covered with powdered paraformaldehyde which was packed closely around them. The watch crystals were left uncovered and placed in a dry place. At

intervals 25 seeds were removed and germinated, with the results shown in Table VI.

TABLE VI.—*Percentage of germination of wheat kept in contact with powdered paraformaldehyde* [a]

Length of contact.	Experiment 1, Little Club, harvester-thrashed.	Experiment 2, Little Club.		Experiment 3, Early Baart, hand-thrashed.	Experiment 4, Early Baart, hand-thrashed.	
		Harvester-thrashed.	Hand-thrashed.		Uninjured.	Seed coats broken over embryo.
1½ hours	80					
24 hours	50	50	90	100	90	10
2 days		30	90	80	80	0
3 days	20					
5 days					80	0
6 days		0	70	30		
8 days					70	0
14 days	10	0	0	0		
20 days					30	0
42 days					20	0
Control	100	100	100	100		

[a] No germination tests were made at the intervals represented by blank spaces.

The data in Table VI show that dry paraformaldehyde powder kills seed in contact with it, even those with unbroken seed coats. Those with the testa injured, either by the thrashing machine or by breaking in the laboratory with a needle, were injured and killed most quickly, as was to be expected. It is noteworthy that the appearance and progression of the seed injury was similar to that previously noted as occurring in the successive germinations of treated seed being dried. The first sign of injury was the retardation of the development of the plumule, which became gradually more extreme. Finally, it was so injured that it did not elongate at all after emerging from the seed, the sheath breaking prematurely and showing the same curved, sickle-shaped deformity previously found so characteristic of dried formaldehyde-treated seeds. As it would be difficult to conceive of any absorption of solid paraformaldehyde, the only plausible explanation of such "paraformaldehyde injury" is the penetration of formaldehyde gas through the seed coat, the gas being concentrated in the interstices of the powder as a result of the evaporation of the latter. Later experiments in which it was found that absolutely dry seeds were uninjured by formaldehyde fumes make it appear probable that the gas is dissolved in the cells of the seeds and really diffuses into them as a solution.

HUMIDITY AS THE DETERMINING FACTOR IN SEED INJURY

The first hint that the humidity of the atmosphere surrounding the seeds at the time of drying determined the amount of seed injury from treatment with formaldehyde—by controlling the evaporation of the

solution on the seed and the formation of paraformaldehyde—came from the difference in the degrees of injury sustained by the original samples of treated wheat dried in the greenhouse, laboratory, and refrigerator (see Tables I and II and Pl. 37). The dried seed from the greenhouse, where the atmosphere was warmest and most humid, was the least injured. From our knowledge of the unstable constitution of paraformaldehyde it seemed probable that it would form but slowly if at all in the presence of moisture. Work, therefore, was undertaken to determine whether the degree of this seed injury resulting from drying after treatment depended on the humidity of the atmosphere at the time of drying.

The moisture content of the three dried samples of treated seed from the greenhouse, laboratory, and refrigeratorw as determined after six weeks of storage. By drying the seed to constant weight in an electric oven at a temperature of 95° C. it was found that the seed dried in the laboratory contained 13.28 per cent moisture, that from the refrigerator 15.35 per cent, and that from the greenhouse 16.63 per cent. Samples of each lot were then tested qualitatively by means of Tollen's silver-mirror aldehyde test for the presence of formaldehyde. A distinct difference was obtained. The precipitate appeared most rapidly and was most dense in the laboratory-stored seed which had the small moisture content, while it was decidedly least in the greenhouse-stored sample with highest moisture percentage. These facts then suggested that the formation of paraformaldehyde is dependent on the dryness of the atmosphere. Since all evidence points to the fact that seed injury upon drying after treatment is dependent on the formation of paraformaldehyde on the seeds as the solution evaporates, it follows that seed injury may vary inversely with the moisture content of the surrounding atmosphere. So far as the three seed lots of this original experiment were concerned, this was true, for the greenhouse where least injury occurred was most humid and the laboratory where injury was most extreme was driest. However, more evidence was necessary, and this could be obtained only by storing treated seed under controlled and definitely known moisture conditions.

Atmospheric humidities varying by 10 per cent intervals from saturation over water to dryness over concentrated acid were produced in desiccators by the use of sulphuric acid dilutions.[1] Given the specific gravity of the solutions necessary to produce the desired atmospheres (Pl. 38, B), they are easily made up in quantity by means of specific gravity spindles and kept in stock bottles (*17, p. 114*).

[1] Since these experiments were completed, a paper written by Neil E. Stevens (*15*) has come to the writer's attention in which a table is given showing the approximate humidities obtained in desiccators containing aqueous solutions of sulphuric acid of various specific gravities. These differ somewhat from those given by Woodworth (*17, p. 114*), and the method is described more fully and the data given are more complete.

Some of the same machine-thrashed Little Club seed used in all these experiments was treated with a 0.1 per cent solution, and, after the surplus liquid was removed by spreading on towels for a half hour, the seed was divided into 11 lots, each lot nearly filling a rectangular glass dish 6 by 8 cm. and 3 cm. deep. One of these dishes of wheat was then placed in each of the 11 desiccators containing 100 cc. of their respective sulphuric acid and water mixtures. These solutions were changed at the end of the first, second, third, fifth, and tenth days, so that they were kept at the proper strength. Samples of wheat were removed after various intervals, and the injury was determined by germinating on blotters at room temperatures.

TABLE VII.—*Relation between seed injury from drying after treatment with a 0.1 per cent formaldehyde solution and the humidity of the atmosphere*

Specific gravity of sulphuric acid and water mixtures.	Approximate percentage of humidity produced in desiccators (20° C.).	Percentage of germination after storage in desiccators for—									
		1 day.	2 days.	5 days.	7 days.	10 days.	16 days.	22 days.	26 days.	28 days. [a]	42 days.
1.000	100	96	98	94	98	(b)	(b)	(b)	(b)	(b)	(b)
1.070	90	98	98	96	96	(b)	(b)	(b)	(b)	(b)	(b)
1.130	80	96	94	92	94	94	96	(b)	(b)	(b)	(b)
1.206	70	94	98	90	90	96	90	98	90	88	100
1.273	60	96	96	.....	90	84	96	90	96	86	92
1.334	50	96	96	.....	82	84	88	86	92	94	88
1.400	40	98	98	.....	70	82	74	78	84	74	82
1.470	30	98	96	.....	74	76	78	70	84	72	76
1.530	20	96	94	74	84	76	80	84	82	70	72
1.604	10	94	98	84	80	80	84	72	88	72	76
1.840	0	96	92	84	88	88	88	82	84	64	86
Control	.....	96	100	98	98	100	98	96	98	100	96

[a] Germinated in soil.
[b] Attacked by molds.

A study of these germination percentages reveals several most interesting facts. It is at once obvious that they show the existence of a close relationship between the seed-treatment injury caused by drying and the humidity of the atmosphere. They show that there is no injury in the damper atmosphere of 70 per cent humidity and above, so long as the seed is not attacked by molds. They show also that there is less injury in the dryest desiccators, those containing from 20 per cent moisture to none at all, than in those of intermediate humidities. These comparative injuries are made clearer by a graph (fig. 2) the points on which represent the averages of all the percentages obtained for each sample, beginning with those obtained after five days' storage. The data for the

samples in 80, 90, and 100 per cent humidities were not included because of the small number of germinations obtained before the seed was partially destroyed by molds. The curve shows graphically that there was a decrease in germination from the uninjured samples in the high humidities to those in 30 per cent humidity, after which it increased in the successively drier desiccators but did not reach normal.

It is also noteworthy, in connection with the data given in Table VII, that no injury appeared, as indicated by the germinated samples, until at some time between two and five days after treatment. Thus, a test of all samples after three days, the results of which were not included in the table because of complications from an unusual growth of Rhizopus in the germinators, showed no visible evidence of formaldehyde injury. The harmful effects were first apparent after five days' storage, where, however, molds again interfered with the germination of four of the samples.

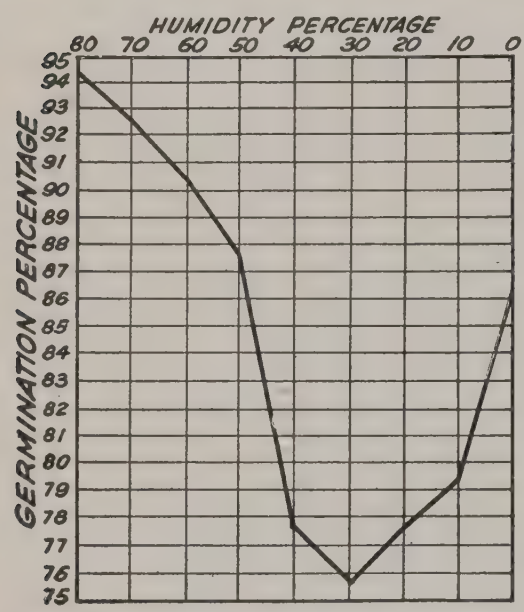


FIG. 2.—Graph showing the relation of humidity of the air to percentage of germination of stored seed in first experiment.

It will also be noticed in Table VII that the successive percentages obtained show no increasing injury between the 5-day and 42-day germinations. They differ in this from those of many other experiments (Tables I to IV).

Some months later this experiment was repeated with some of the same lots of wheat. This second experiment differed from the first, so far as was known, only in the smaller quantities of treated wheat placed in each desiccator and in the amounts of sulphuric acid and water mixtures used. Approximately 20 cc. of wheat were put in each desiccator, which was about one-fourth of the quantity used before. One hundred cc. of the desiccating solutions were left in the desiccators for the first 24 hours, at the end of which period they were changed, and 200 cc. quantities of the fresh solutions were substituted and left unchanged for the rest of the experiment. As will be seen from Table VIII, the resulting seed injury was more extreme than in the first experiment and reached its maximum in a more humid atmosphere (Pl. 39). The explanation for the difference may be the greater or lesser effectiveness of the desiccating solutions, owing to the difference in the quantities used and in the amount of seed dried over each.

The data in Table VIII show, as do those of the preceding experiment, that the highest humidities allow no injury and that in the lowest the germination percentages are normal also, only the retarded growth giving

evidence of some deleterious effect of the treatment. There is a very definite point of maximum injury—the 70 per cent humidity. This is somewhat different from the situation in the preceding experiment, where the maximum injury was at approximately 30 per cent humidity, with none at all occurring at 70 per cent.

TABLE VIII.—*Data from the second experiment on the relation between humidity and seed injury after formaldehyde treatment*

Specific gravity of sulphuric acid and water mixtures.	Approximate percentage of humidity produced in desiccators (20° C.).	Stored 10 days.		Stored 21 days.		Stored 35 days.		Stored 42 days.	
		Germination.	Height of plants.[a]	Germination.	Height of plants.[a]	Germination.	Height of plants.[a]	Germination.	Height of plants.[a]
		Per ct.	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*
1.000	100	96	5	98	8.0	.....	.....	.....	.....
1.130	80	94	5	100	7.0	.....	.....	.....	.....
1.206	70	6	1—	4	1.0—	0	.....	12	1.0—
1.273	60	18	1—	38	1.0—	20	.....	12	1.0—
1.334	50	96	2	70	1.0	45	.....	88	1.5
1.400	40	90	4	84	1.5	80	.....	92	1.5
1.530	20	90	5	100	5.0	90	.....	96	3.5
1.604	10	98	5	98	4.0	80	.....	88	3.5
1.840	0	96	5	98	6.0	100	.....	88	3.5
Control	.....	98	5	98	7.0	100	.....	88	6.0

[a] The average heights of the plumules after 6 days are given for each germinating sample, because a comparison of these for all the samples of any one test shows any injury indicated by retardation which sometimes would not be shown by the germination percentage alone. A height of less than one centimeter (1—) indicates extreme injury, with usually stunted, deformed plumules which could not reach the surface of the soil.

Figure 3 shows more plainly the comparative germinations given in Table VIII. As in figure 2, each point was obtained by averaging all the germination percentages given by the sample stored at each indicated humidity.

Since all germinations were made in blotters without temperature or humidity control, the rate of growth of seedlings of successive 6-day germinations of the same sample varied in a meaningless way and so were valueless except for comparisons of the injury shown by the different samples in the same germination test. However, as noted in the discussion of the first experiment with the desiccators, the growth measurements follow closely the germination percentages and are more delicate indicators of harmful effects of treatment than the latter.

If the averages of the heights of the seedlings from each desiccator for all the germination tests of both experiments be plotted with the humidities in which the respective seed samples were stored, a graph such as figure 4 is obtained. These heights were measured after six and seven days' growth, but the conditions of germination in successive

tests were so variable that accurate comparisons of growth can not be made. However, the graph, in its similarity to the germination graphs, illustrates the close correlation between viability of the sample and the retardation of seedling growth. Being an average of the two experiments, it brings the maximum growth retardation to 60 per cent.

Since the relation between degree of seed injury and the moisture content of the atmosphere in which the seed was stored had been shown, it was surmised that a similar correlation could be shown to exist between humidity and the formation of paraformaldehyde. After the seed samples of the first experiment were removed from the desiccators, a Syracuse watch glass containing 10 cc. of commercial formaldehyde solution was placed in each. The solid polymer first appeared after three days as a white suspension in the dishes in humidities of 20 and 10 per cent, and in the very dry atmosphere over concentrated acid. Two days later only the dry, white solid was left in these dishes, and a white precipitate made the solutions opaque in the 30, 40, and 50 per cent atmospheric humidities. The density of the suspensions was in inverse proportion to the humidity in these desiccators. Not until 10 days had passed did any paraformaldehyde appear in the 60 per cent humidity, at which time all that formed earlier in the dishes in the dryer atmospheres was dry. No sign of the white solid ever appeared in the more humid desiccators, although the solution in 70 per cent eventually evaporated to dryness (Pl. 38, B). It was very interesting thus to find that the highest humidity permitting the formation of paraformaldehyde was also the highest in which seed injury occurred after treatment with the 0.1 per cent solution of formaldehyde—that is, the germination of wheat was lowered in the same desiccators in which paraformaldehyde formed upon the evaporation of formaldehyde solutions in them.

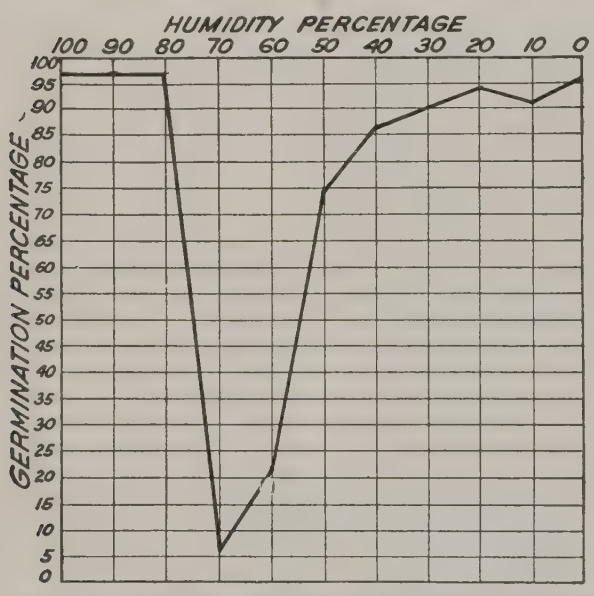


FIG. 3.—Graph showing the relation of humidity of the air to percentage of germination of stored seed in second experiment.

Again, at the end of the second experiment, after the wheat was removed from the desiccators, dishes containing equal quantities of undiluted formaldehyde solutions were placed over the sulphuric acid dilutions, and the appearance and rapidity of formation of paraformaldehyde were noted. In this case 5-cc. instead of 10-cc. quantities were used. After two days, the first white suspension appeared in the desic-

cators having humidities varying from 40 per cent to dryness, being very faint in the former and increasing to a considerable quantity in the latter. The next day a faint opaqueness showed in the dishes of solution in the 50 per cent, and on the day following in those in the 60 per cent humidity, at which time all those in the drier chambers were entirely dry. It is indeed interesting that although no solid ever formed in the 70 per cent humidity, this dish, as in the preceding experiment, evaporated to dryness but left no residue. The volume of liquid left unevaporated in the dishes in the damper atmospheres was greater the higher the humidity (Table IX).

When the residue of paraformaldehyde left after the evaporation of the solutions in the drier desiccators was weighed, it was found in both experiments that, in general, the quantity formed varied inversely with the humidity of the atmosphere (Table IX). Since the degree of injury to the stored treated wheat was in the opposite order, it was at once evident that the factor causing the progressive variation in seed injury in the desiccators was not the quantity of paraformaldehyde formed on the seeds. Before this point is considered further, however, the results of a contemporaneous experiment should be presented. When the dishes of formaldehyde solution were placed in the desiccators to be evaporated, small quantities of untreated seed were inserted at the same time to determine if formaldehyde gas would evaporate in each humidity to produce sufficient concentrations in the different atmospheres to kill the wheat exposed to them. When samples of this wheat were germinated at the end of the experiment, surprising results were obtained. It was found after both experiments that there was no germination of this seed from desiccators of 70 per cent humidity and above and that the germination percentages of seed from the drier atmospheres varied inversely with the moisture percentage, the seed being least injured by the formaldehyde fumes from the solution over concentrated acid. All these secondary experiments on the dependence of the behavior of formaldehyde and its solutions on atmospheric humidity are summarized in Table IX.

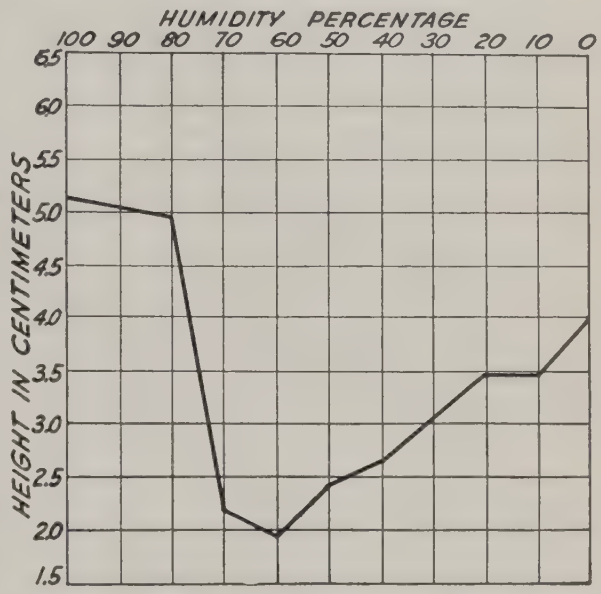


FIG. 4.—Graph showing the relation between humidity of the air and seed injury as indicated by rate of growth of germinated seedlings.

In brief, then, the facts are these: The seed injury resulting after treatment with a 0.1 per cent solution, which occurs as the result of drying

in atmospheres of such moisture content as permit the formation of paraformaldehyde in evaporating solutions, is greatest in intermediate humidities, becoming less as the moisture percentage decreases. This is in spite of the fact that there is an increase in the quantity of paraformaldehyde formed in these successively lower humidities. Secondly, the degree of injury to untreated seed placed in desiccators alongside of evaporating formaldehyde solutions in closed chambers is least in the driest atmosphere and increases with increased humidity. It therefore seems probable that the seeds in the lower humidities were so dry that penetration of the seed coat by formaldehyde was difficult because of the lack of sufficient moisture to permit solution of the gas on or in the testa and its subsequent diffusion to the embryo.

TABLE IX.—*Relation of the humidity of the atmosphere to the evaporation of formaldehyde solutions, the formation of paraformaldehyde, and the effects of the fumes on untreated wheat*

Humidity.	Length of time before appearance of paraformaldehyde in the solutions.		Weight of paraformaldehyde formed.		Volume of solution left unevaporated.		Germination of untreated wheat left in desiccators during evaporation of formaldehyde.	
					After 40 days.	After 24 days.		
	Exp. 1 (10-cc. quantity).	Exp. 2 (5-cc. quantity).	Exp. 1 (10-cc. quantity).	Exp. 2 5-cc. quantity).	Exp. 1 (original volume 10-cc.).	Exp. 2 (original volume 5-cc.).	Exp. 1 (with 10-cc. quantity).	Exp. 2 (with 5-cc. quantity).
Per cent.	*Days.*	*Days.*	*Gm.*	*Gm.*	*Cc.*	*Cc.*	*Per cent.*	*Per cent.*
100	.....	.....	.....	.....	9.6	5.0	0	0
90	.....	.....	.....	.....	7.2	.....	0	0
80	.....	.....	.....	.....	5.4	1.8	0	0
70	.....	.....	.....	.....	.0	.0	0	0
60	10	4	0.07	0.07	.....	.....	0	0
50	5	3	.40	.96	.....	.....	0	0
40	5	2	1.33	1.14	.....	.....	0	0
20	3	2	2.25	1.36	.....	.....	4	10
10	3	2	1.12	1.42	.....	.....	36	16
0	3	2	1.12	1.36	.....	.....	54	30

In presenting this explanation, we are assuming that formaldehyde does not penetrate seed coverings easily, if at all, as a gas but must be dissolved. A small quantity of moisture in the cells of the seed covering therefore would perhaps be necessary to permit injury from formaldehyde fumes. This is consistent with the statement of Humphrey and Potter (7) that—

> disinfection with formaldehyde gas seems to require some moisture.

This supposition would explain the relation found between the degree of injury resulting from drying treated seed and the humidity of the atmosphere in which the seed is dried. With the atmosphere sufficiently dry to allow the formation of the "formaldehyde reservoir"—the coating of paraformaldehyde on the seed—the ease of penetration of the formalde-

hyde gas constantly formed next to the seed by its decomposition would be determined by the moisture in the seed coat. It would follow, as was actually found, that there would be a point where maximum seed injury would occur—at a humidity low enough to permit the solid polymer to form on the seed as the solution evaporates, yet high enough to permit diffusion in solution of the gas formed from it through the cells of the seed coat to the embryo. Thus may be explained the gradual lessening of the degree of injury from the point of maximum injury to practically normal germination in dry atmospheres.

The work of Arcichovskij (*1*) on the effect of graded concentrations of formaldehyde solutions ranging from 0.125 to 40 per cent supports the assumption that the ease of penetration of formaldehyde is dependent on the dilution of the solution as it passes through the cells into the seed. He found that, for any given duration of exposure, seed injury did not increase directly with the concentration of the solution. After a definite point of maximum injury, the harmful action of the solution decreased with increased concentration, until in all exposures over four hours the undiluted 40 per cent formaldehyde solution caused less injury than the 0.125 per cent dilution. For instance, after 256 hours 37.5 per cent of the seeds from the 40 per cent solution germinated, while those in the 0.125 per cent solution were entirely killed after 32 hours' exposure. The curve he has drawn showing the relation between concentration of the solution and the percentage of germination is similar to the curves in this report which show the relation between humidity and formaldehyde injury to seeds upon drying after treatment.

The preceding paragraphs merely offer a suggestion of an explanation of the observed facts. This interpretation of these facts is based on several assumptions which have not been proved by direct evidence. One is that paraformaldehyde, as a solid, does not injure seeds but only upon its breaking down into formaldehyde gas and forming a toxic vapor about the seed. Another is the assumption that this formaldehyde does not penetrate seed coats as a gas but that it must enter in solution.

It should be pointed out here that in experiment 2 the maximum injury occurred in the atmosphere of 70 per cent humidity (Table VIII) in the desiccator in which it was found that the formaldehyde solution evaporated to dryness without the formation of paraformaldehyde (Table IX). This indicates that seeds may be injured by the concentration of a 0.1 per cent solution on the surface as evaporation proceeds, without the formation of the solid polymer.

RELATION OF DEGREE OF INJURY TO MANNER OF DRYING

In the course of the experiments it was noted that the drying injury was not always of the same severity, and it was finally found that it depended on the aeration of the drying sample, thinly spread seed escaping the injury suffered by that dried in heaps. This observation was

decided to be consistent with our previous conclusions as to the manner in which formaldehyde solutions injure the treated seeds upon which they dry. If injury occurred as the result of the close adherence to the seed of concentrated formaldehyde gas formed by the decomposition of paraformaldehyde deposited on the surface as the seed dried, then it would follow that well-aerated seeds might very probably escape injury by virtue of the rapid breaking down of the polymer and its escape by diffusion into the air. Formaldehyde gas is heavier than air, so that if seeds were dried in large quantities in sacks or in boxes, diffusion would be slow and the air around the seeds would become saturated with gas, which would be held around them long enough to cause seed injury.

The evaporation of but a relatively small quantity of paraformaldehyde in a closed space saturates the atmosphere so that further breaking down

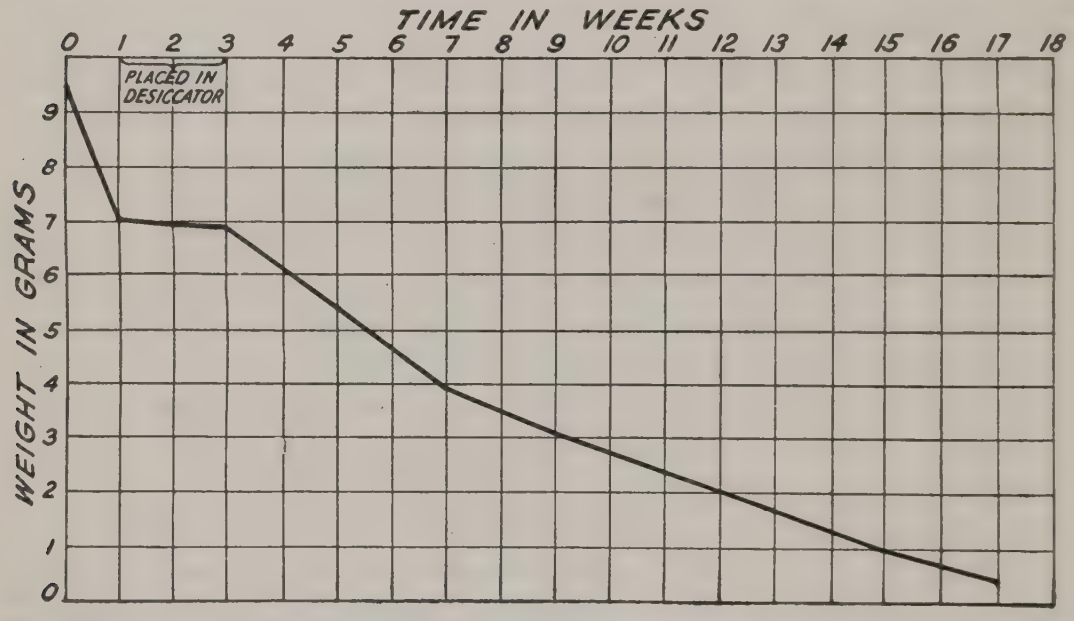


FIG. 5.—Graph showing the diminution in the rate of evaporation of paraformaldehyde inclosed in a desiccator of 2,400-cc. volume.

of the solid is inhibited by the partial pressure of the formaldehyde gas. This was shown experimentally by placing some paraformaldehyde in desiccators at the same time that dishes containing approximately the same quantities were evaporating in the open air of the room. The rate of evaporation of each sample was measured by the loss in weight after successive weekly intervals. Figure 5 illustrates the initial rapid rate of evaporation of a sample in the open air and the slowing up of that rate when it was placed in a 2,400-cc. desiccator containing calcium chlorid as a drying agent. When the sample was removed from the desiccator the rate increased again, and the curve representing this period shows a steady, even fall, until after 18 weeks the solid had practically disappeared. If we compare the curve with figure 1, we note that whereas when the sample is exposed to the open air it disappears entirely, when it is inclosed and hence unaerated its evaporation practically stops. The exact weight

of the solid which when evaporated in a space of 2,400 cc. checked by its partial pressure further decomposition of the sample is not shown. It would appear to be approximately 0.1 gm., the average decrease in weight found upon successive weekly weighings of the inclosed sample. The slight fall of the curve for this period in the desiccator is explained by the fact that when the dish was removed each time for weighing the concentration of gas within would be diluted and so the sample would continue to lose weight. A parallel control experiment gave the same curve and the same total loss in weight, 0.21 gm., during the two weeks in the desiccator.

The significance of this curve for the problem of post-treatment injury of dried seeds is that when there is no aeration the formaldehyde gas from the evaporating paraformaldehyde on the seeds easily saturates the atmosphere in the interstices of the sample and inhibits the evaporation of more of the solid. The slower the outward diffusion of the gas the longer will the paraformaldehyde remain on the seed surfaces and the longer will a toxic atmosphere exist about them. As the penetration of the seed coat and subsequent injury by formaldehyde is comparatively slow, usually occurring in from 3 to 5 days with a 0.1 per cent solution (Table VII), it is entirely conceivable that with rapid drying and thinly spread seed any paraformaldehyde formed can be completely evaporated and its dissipation effected so rapidly that it can not enter and injure the embryo.

Seeds treated with a 0.2 per cent solution, twice as strong as the usual treatment, were dried without injury when spread in a single layer on towels, while such seeds dried in quantity in an open box were practically all killed. That it was the time required for the formaldehyde to penetrate the testas which saved the former lot of seed was shown by the fact that some of the same sample which had the seed coats broken over the embryos were dried beside the others and were severely injured after 24 hours. In the former case the paraformaldehyde evaporated and diffused before it could penetrate the sound seed coat. But when a 4.5 per cent solution was used, even the seeds with unbroken coats were found to be injured after 24 hours' drying under these conditions. The quantity of paraformaldehyde formed presumably was too great to escape before seed injury occurred. The broken seeds dried at the same time showed proportionately greater and more rapid injury than the broken seeds treated with the weaker solutions. It will be noted in Table X that embryos exposed by broken testas are not injured by a 10-minute dip into formaldehyde as strong as 0.2 per cent but that a 4.5 per cent solution is injurious. It is significant that with rapid drying and aeration even the seeds with broken seed coats were not injured by a 0.1 per cent solution. Yet it has been found repeatedly that when perfect seeds thus treated are dried without aerating they are injured or killed.

TABLE X.—*Relation between strength of formaldehyde solution, condition of seed coat, and the cumulative injury to Early Baart wheat well spread during the drying period*[a]

Length of drying period.	4.5 per cent formaldehyde solution.				0.2 per cent formaldehyde solution.				0.1 per cent formaldehyde solution.			
	Seed coats unbroken.		Seed coats broken over embryo.		Seed coats unbroken.		Seed coats broken over embryo.		Seed coats unbroken.		Seed coats broken over embryo.	
	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.
	Per cent.	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*
0	100	5.0	60	2.5	95	5.0	95	5.0	95	5.0	90	4.5
1¼ hours	95	2.5	30	1.0—	95	3.5	95	2.5	100	4.0	100	4.0
4 hours	95	2.5	40	1.0—	95	3.5	90	2.5	100	5.0	100	5.0
24 hours	95	2.5	25	1.0—	100	4.0	80	3.0	100	5.0	90	5.0
3 days	75	2.0	20	1.0—	95	2.0	45	1.0	100	5.0	95	5.0
6 days	80	1.0	15	1.0—	95	2.5	45	1.5	100	5.0	95	3.0
14 days	65	1.0—	0	1.0—	95	2.0	10	1.0	90	3.5	100	3.5

[a] The average heights of the plumules after 6 days are given for each germinating sample, because a comparison of these for all the samples of any one test shows any injury indicated by retardation which sometimes would not be shown by the germination percentage alone. A heighth of less than one centimeter (1–) indicates extreme injury, with usually stunted, deformed plumules which could not reach the surface of the soil.

In brief, Table X shows that when treated seed is dried rapidly by being thinly spread in the laboratory, it is uninjured by a 0.1 per cent solution even if the embryos are exposed by broken seed coats; that seed treated with a 0.2 per cent solution is uninjured if the seed coat is perfect, but severely injured after 24 hours if it is broken; and that, with a 4.5 per cent solution, perfect seeds are slowly injured and that seeds with broken testas are injured by the dip into the treating solution, which injury rapidly increases upon drying. The cumulative nature of this seed injury is well shown by the germination data for all these injured samples.

Lest there be any misunderstanding, it may be well to consider again the case of treated seed which is sealed damp. It may be asked at this point that if aeration is necessary to prevent injury from formaldehyde fumes, how can seed stored damp in sealed jars remain uninjured? The answer is probably to be found in the fact that paraformaldehyde does not form on damp seeds; hence the damp seeds are not surrounded by concentrated formaldehyde vapor. The moisture in the jar is a weak dilution, and neither it nor the amount of formaldehyde in the air in the presence of so much water is strong enough to injure the seed. Moreover, the formaldehyde does not remain on damp seeds indefinitely, owing to the activity of microorganisms which decompose it. The case is different with solutions stronger than 0.1 per cent, however. Damp seed is slightly injured by a 0.2 per cent solution after 24 hours' storage, and a 4.5 per cent solution is fatal in a sealed jar. Whether in these instances it is the solution on the seed which injures or the resulting

formaldehyde fumes was not determined; but according to Auerbach and Barschall (*2*), the partial pressure of formaldehyde gas above solutions in a closed space increases with the concentration of the formaldehyde solution; hence the fumes may be the cause of injury.

Several experiments showed clearly the varying degrees of injury resulting from drying the seed at different rates. The usual procedure was to treat some wheat with a 0.2 per cent solution and some barley with a 4.5 per cent solution, the latter being more resistant to drying injury and therefore requiring the use of a strong solution to produce it. Some of each lot was then spread thinly over towels on the laboratory table, while the rest was put in an open tumbler or a slender, uncovered bottle. For comparison, a third lot usually was placed in a similar bottle and sealed while damp. Samples were removed after various intervals and were germinated in the usual way to determine the degree of injury. The data on the germination of wheat are shown in Table XI and those on the germination of barley in Table XII.

TABLE XI.—*Percentage of germination of Little Club wheat treated with 0.2 per cent solution of formaldehyde and dried under different conditions and during periods of varying lengths*

Length of drying period	Experiment 1.			Experiment 2.		Control, untreated.
	Spread on towels.	In open bottle.	In sealed bottle.	Spread on towels.	In open bottle.	
Days.						
0	96	96	96	94	94	96
1	84	74	82	94	76	98
2	.....	.....	.....	98	88	96
3	70	52	84	.....	.....	100
6	64	40	80	76	64	98
18	40	16	82	88	52	98
28	.....	.....	.....	74	52	94
60	50	8	80	56	50	92

Wheat treated with a 0.1 per cent solution was dried overnight in a sealed jar, in an open jar, and in a thin layer on towels. After drying 24 hours, equal samples were washed in equal volumes of water, and the washings were subjected to Tollen's aldehyde test for the presence of formaldehyde. Comparison of the density and rapidity of formation of the silver precipitate showed that there was least formaldehyde on the thinly spread seed and greater amounts on the other two samples. At the end of the second 24-hour period the experiments were repeated. It was found that the amount of formaldehyde on the sealed seed had diminished until it gave a much less dense precipitate than either of the dried samples. Of the latter, the extract from the seed dried in the bottle showed the presence of more formaldehyde than that from the

well-spread seed. Throughout subsequent tests, continued almost daily for two weeks, the dried samples gave stronger reactions than the damp ones, which, after about six days, showed no more than the extract from the untreated control. The dried samples soon gave about equal reactions. The results of the first two tests, which showed that there was more formaldehyde on the seed dried in the open bottle than on that spread on towels, confirm the conclusion already drawn from the germination data—that is, that more paraformaldehyde remained on the seed dried without aeration because the formaldehyde gas could not escape readily from around the seed. Gradually, however, this gas escaped and the quantity present, as shown by the reaction, decreased to that of the aerated sample.

TABLE XII.—*Percentage of germination of Coast barley treated with 4.5 per cent formaldehyde solution and dried under different conditions and for varying periods of time*

Length of drying period.	Experiment 1.				Experiment 2.		
	Spread on towels.	In open bottle.	In sealed bottle.	Control, untreated.	Spread on towels.	In open bottle.	Control, untreated.
Days.							
0	98	98	98	98	80	80	88
1	88	66	64	94	86	74	90
2	.....	.....	.....	.....	82	44	92
6	80	20	4	94	50	0	92
17	68	0	0	90	82	10	96
28	36	0	0	84	52	2	92
42	[a] 80	8	4	96	.....	.....	.....
60	.....	.....	.....	.....	[a] 70	10	90

[a] These increased germinations after 42 days, though they apparently indicate recovery, are probably due to more favorable germination conditions.

From these data it appears that any prediction or explanation of post-treatment injury must be based on the humidity of the atmosphere immediately surrounding the seed and on the manner of drying the seed as affected by its aeration. Temperature may also be an important factor, but its relation to the problem has not yet been determined. Temperature or some other variable must account for the fact that, with all the foregoing conditions controlled, repetitions of experiments do not always give the same results. For instance, in Table XI injury is shown to thinly spread wheat after a subjection to a 0.2 per cent solution of formaldehyde, while less injury is shown in the data in Table X. In these cases the differences may possibly be chargeable to the fact that different samples of different varieties of wheat were used. In one experiment no greater injury occurred to seed dried in an open bottle than to that thinly spread on the table beside it. Such exceptions are only occasional, but they indicate that certain apparently minor factors have not yet been ascertained.

TABLE XIII.—*Germination and seedling growth of Little Club wheat treated with 0.1 per cent formaldehyde solution and sealed in bottles after drying during various periods* [a]

Length of storage period after drying.	Sealed at once after treatment, 21.20 per cent moisture.		Dried 1 hour, 20.26 per cent moisture when sealed.		Dried 10 hours, 17.08 per cent moisture when sealed.		Dried 20 hours, 14.50 per cent moisture when sealed.		Dried 30 hours, 13.81 per cent moisture when sealed.		Dried 48 hours, 12.49 per cent moisture when sealed.		Dried 72 hours, 12.59 per cent moisture when sealed.	
	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.
Days.	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*
0 (end of drying period).....	100	4·5	98	4·5	100	4·5	98	4·5	100	4·5	100	4·5	100	4·0
6.....	100	3·5	98	4·0	84	1·0—	92	1·0—	90	1·0—	94	1·0	98	3·0
12.....	100	5·0	100	5·0	76	1·0—	88	1·0—	98	1·0—	100	3·0	98	4·5
21.....	98	4·5	98	4·0	72	1·0—	52	1·0—	68	1·0—	98	1·5	94	1·5

[a] The average heights of the plumules after 6 days are given for each germinating sample, because a comparison of these for all the samples of any one test shows any injury indicated by retardation which sometimes would not be shown by the germination percentage alone. A height of less than one centimeter (1—) indicates extreme injury, with usually stunted, deformed plumules which could not reach the surface of the soil.

Interesting data were obtained as the result of an experiment originally intended to show the relation between the moisture content of the seed and the degree of injury upon drying. Samples of wheat and barley were treated with a 0.1 per cent solution for 10 minutes, drained 10 minutes, and allowed to dry partially by spreading on towels for an hour. At the end of that time about 70 cc. were sealed in a small screw-top bottle, and the rest were allowed to continue drying. Equal quantities were removed from the drying lot daily for four days and sealed, the object being to get samples of different moisture content so stored as to insure constant humidity in the bottles. It was found that constant weight was reached after two or three days' exposure to laboratory air. The moisture percentage of each sealed sample was obtained by drying in an electric oven at 95° C. Samples from each bottle were germinated after various intervals, and the injury shown by each was compared by means of germination percentages and rate-of-growth observations. Tables XIII and XIV summarize the results of several experiments.

The data in Table XIII show that none of the samples were injured by the drying period which preceded their being sealed. This was due, no doubt, as explained earlier in this paper, to the fact that they were spread thinly and, therefore, were well aerated. In the second place, it shows, surprisingly enough, that the subsequent injury from being sealed did not bear a direct relation to the moisture content of the seed, as had been expected. After 21 days' storage samples sealed wet immediately after treatment and those sealed after 1 hour's drying were uninjured. This was to be expected, for they contained too much moisture to permit the formation of paraformaldehyde. But in all germination tests made after 6 or more days' storage those samples dried for 10, 20, and 30 hours before sealing showed extreme injury, while those dried longer were less injured. Seed dried 72 hours before sealing was nearly as free from injury as the uninjured, damp seed. This lesser injury to the samples dried for the longer periods seemed so puzzling that the experiment was repeated, twice with wheat and once with barley, with the same results.

The data in Table XIV again show that, although no injury resulted from these various drying intervals, yet when the seed was sealed there was extreme injury after 5½, 9, and 24 hours' drying, after 48 hours slight injury (retarded plumules), and after 72 hours practically no injury. The maximum injury occurred in seed dried 5½ and 9 hours, respectively, decreasing steadily with the longer drying periods of the other samples. This shows particularly well in the germinations of seed in soil, where the weak and injured seedlings, called "germinated" on the blotters, did not reach the surface of the ground and so particularly emphasized the injury to the 5½- and 9-hour samples. Elsewhere in this paper it has been noted that blotter germinations suffice to show comparative injuries and to indicate the deformity and retardation of the seedlings; but,

except to one trained to distinguish the weakened and injured seedlings, the germination counts will not give an accurate measure of field results. In soil, the percentage of germination of injured samples will be much lower, of course, depending on the nature of the soil and the difficulty encountered by the seedling in emerging from it.

TABLE XIV.—*Percentage of germination of Little Club wheat and Coast barley treated with 0.1 per cent formaldehyde solution and sealed in bottles after drying for various periods*

LITTLE CLUB WHEAT

Length of storage period after drying.	Dried 1 hour, 20.96 per cent moisture when sealed.	Dried 5½ hours, 18.36 per cent moisture when sealed.	Dried 9 hours, 16.34 per cent moisture when sealed.	Dried 24 hours, 14.81 per cent moisture when sealed.	Dried 48 hours, 12.94 per cent moisture when sealed.	Dried 72 hours, 13.37 per cent moisture when sealed.	Dried 96 hours, 12.15 per cent moisture when sealed.	Control, untreated, 12.06 per cent, moisture when sealed.
Days.								
1	100	96	88	98	98	100	98	96
7	100	58	60	82	94	94	96	96
7[a]	92	14	2	70	88	100	88	96
14	92	48	42	74	92	92	94	100

COAST BARLEY

1	94	92	88	92	98	100	96	96
7	76	74	68	92	.....	90	78	.....
7[a]	98	44	66	88	96	100	94	96
14	48	26	20	72	42	66	.....	.....

[a] Germinated in soil; all others germinated on blotters.

This second experiment also demonstrated that this phenomenon is shown by barley as well as wheat. For barley, as for wheat, the maximum injury was to those samples dried for 5½ and 9 hours, with decreased injury to the samples dried longer before sealing.

In subsequent experiments on wheat treated with both 0.1 per cent and 0.2 per cent solution, then dried and sealed, there was always this upward gradation in injury from a maximum below 24 hours of drying to almost normal germination in samples dried for several days and then sealed. However, in the experiment illustrated in Plate 40, there was severe, though lessened, injury to the sample dried three days before being sealed in the bottles.

For a long time after the first results of this nature were obtained they seemed inexplicable. After the later studies of the behavior of formaldehyde and the manner in which it injures seeds through the volatilizing of its polymer, paraformaldehyde, an explanation suggested itself. In the first place, it is obvious from what we know of paraformaldehyde that it did not form on the dampest seeds. Hence, those seeds sealed after one hour showed no injury because at the end of that time they were still damp. Paraformaldehyde formed on those dried more thoroughly, and the gas resulting from its evaporation at once began to diffuse away from around the seeds because they were thinly spread.

As a result of this steady evaporation of the paraformaldehyde from these seeds those spread the longest before sealing had the smallest quantity on them when put in the bottles, while those sealed earlier had increasingly greater quantities. Since evaporation of the solid would continue to a certain extent after the seeds were in the bottles, it would seem plausible that the concentration of formaldehyde gas in the atmospheres of the sealed bottles would vary, being greatest where seed had previously dried for but a few hours and least where it had had a longer time to dissipate into the air before sealing. It follows that the seed injury in each bottle is proportionate to the quantity of paraformaldehyde left on the seed at the time of sealing, which, upon evaporation in the bottle, cannot escape and is held around the seed.

SUSCEPTIBILITY OF OTHER GRAINS TO POST-TREATMENT INJURY

In laboratory experiments it was found that barley is much less sensitive than wheat to dry-storage injury after treatment with a 0.1 per cent solution and often escapes injury altogether. Retardation or a slight lowering of the germination percentage usually results, however, from drying the seed in bulk or from sowing it in dry soil. In experiments where the seed was allowed to lie in dry soil for varying intervals one experiment showed rather severe injury, while the two repetitions showed none at all. If a 0.2 per cent solution or a 4.5 per cent solution is used the characteristic cumulative post-treatment injury occurs markedly, just as in wheat. The latter strength is especially destructive when the seed dries (Table XII). The germination percentages shown in Table XV (on blotters, with one exception) are typical of the results obtained in the laboratory when Coast barley was dried in tumblers after treatment.

TABLE XV.—*Percentage of germination shown by Coast barley when dried in the laboratory after formaldehyde treatment*

Length of drying period.	0.1 per cent solution.		0.2 per cent solution.		4.5 per cent solution, Exp. 2.	Control, untreated.	
	Exp. 1.	Exp. 2.	Exp. 1.	Exp. 2.		Exp. 1.	Exp. 2.
½ hour	94	92	92	94	98	96	90
7 days	90	94	74	86	52	90	88
21 days	84	.....	82	.....	.....	90	.....
42 days	84	.....	32	.....	.....	92	.....
56 days	88	80	70	52	6	90	90
70 days [a]	76	88	34	40	2	.....	92

[a] Germinated in soil.

The presence of the glumes on the barley grains probably affords the protection which makes them more resistant than wheat to the harmful effects of treatment and subsequent drying.

Three sorghums, Brown durra, Honey sorgo, and Sudan grass, were found to be uninjured by either a 0.1 per cent or a 0.2 per cent solution of formaldehyde even after weeks of drying. When the seed was stored dry in the same manner as was the severely injured wheat, no effects of the treatment ever appeared. This probably is due to protection afforded by the glumes in some instances, and in others by the thick seed coats.

PREVENTION OF POST-TREATMENT INJURY RESULTING FROM DRYING

McAlpine (*11*) thought that soaking the seed which had been held some time before sowing prevented the appearance of formaldehyde injury, but neither Darnell-Smith and Carne (*5*) nor Kiessling (*9*) was able to confirm this. The writer also has been unable to show that the injury can be avoided in this way. Soaking the seed hastened the germination, as it always does even with untreated wheat. But the characteristic injury to the seedling remained, and the percentage of germination, although occasionally somewhat augmented, was far from normal. It seems probable, therefore, that the hardening of the pericarp is not the primary injury.

It has been shown in this paper that thorough aeration of the treated seed as it dries retards and lessens storage injury but does not always prevent it (Tables X, XI, and XII). Neither is rapid drying possible where large quantities of wheat are handled. However, it was found that dry-storage injury can be entirely avoided by simply washing the seed with water after treatment (Pl. 41). The extent to which this simple procedure would do away with the danger in the use of formaldehyde solutions is shown by the data in Table XVI.

TABLE XVI.—*Percentage of germination of wheat treated with 0.1 and 0.2 per cent formaldehyde solutions and washed with water, compared with percentage of germination of unwashed samples*

Length of drying period.	0.1 per cent solution.		0.2 per cent solution.		Control, untreated.[a]
	Seed not washed in water.	Seed washed in water.	Seed not washed in water.	Seed washed in water.	
Days.					
0	78	78	72	76	70
7	62	74	50	74	72
14	58	74	30	82	76
30	52	.....	32	76	72
60	36	74	8	72	74

[a] This seed had been injured by fumigation with carbon bisulfid, hence the low germination of the untreated control and washed samples.

SUMMARY

(1) No seed injury was produced by treating wheat with either a 0.1 per cent (1 to 40) or a 0.2 per cent (1 to 20) solution of formaldehyde if the seed was germinated immediately after treatment.

(2) If treated seed is held several days or more before sowing, it is severely injured if allowed to dry without thorough aeration during the storage period. If, however, the seed remains damp, it suffers no injury from a 0.1 per cent solution and can be so kept indefinitely or until attacked by molds.

(3) Post-treatment injury is usually cumulative, increasing in degree the longer the seed is stored.

(4) This seed injury upon drying apparently is due to a deposit of paraformaldehyde on the seed, which forms as the formaldehyde solution evaporates. The solid paraformaldehyde, being volatile, is constantly breaking down into formaldehyde gas. This gas, being thus concentrated and held so close to the seed, penetrates it slowly, probably going into solution in the testa.

(5) The degree of post-treatment injury depends primarily on atmospheric humidity during the storage period. In atmospheres damper than 70 per cent humidity the treated seed can be kept indefinitely without ill effects. In those of 70 per cent and less there is decided injury, which is most severe in the intermediate humidities, gradually decreasing in the lower ones until seed stored in an absolutely dry chamber is almost uninjured.

(6) No paraformaldehyde formed upon the evaporation of formaldehyde solutions placed in these damper chambers in which no seed injury occurred, but it did form in all solutions evaporated in desiccators of 60 per cent humidity and less, the quantities by weight increasing as the atmosphere became drier. Therefore, seed injury in the desiccators was not determined by the quantity of paraformaldehyde formed on the seeds in each.

(7) Untreated wheat, when placed in desiccators of varying atmospheric humidities alongside of evaporating, undiluted 36.2 per cent formaldehyde solutions, was least injured in the absolutely dry chamber and was entirely killed by the formaldehyde vapor in all the chambers damper than 30 per cent humidity.

(8) In view of the facts that treated seed is less injured in very dry atmospheres than in intermediate ones and that untreated seed is least injured by formaldehyde fumes in the dry atmosphere of desiccators, it is considered probable that formaldehyde does not enter seeds as a gas or in the solid polymeric form but in solution in the seed coats. For the maximum seed injury to occur as a result of drying after formaldehyde treatment, therefore, there must be an optimum atmospheric humidity

to permit, first, the formation of paraformaldehyde, and second, the solution of formaldehyde gas in the seed.

(9) This post-treatment injury is minimized by spreading the seed as it dries so that maximum aeration occurs, thus hastening the evaporation of paraformaldehyde and the escape of the gas from around the seed.

(10) Barley is less susceptible to post-treatment injury upon drying after soaking in a 0.1 per cent solution, probably because of the protection afforded by the glumes; but when stronger solutions are used the injury is very severe.

(11) Seed dried for an hour by being thinly spread on towels in the laboratory and then sealed in bottles is uninjured after weeks of storage; but seed dried longer, although uninjured by the rapid drying, is injured upon being sealed, presumably because of the concentration of gas in the bottle as a result of decomposition of the paraformaldehyde on the seed. Treated seed dried from 5 to 24 hours was more injured upon being sealed than when dried for a longer time.

(12) The sorghums, Brown durra, Honey sorgo, and Sudan grass, are uninjured upon being stored dry after treatment, even when a 0.2 per cent solution is used.

(13) Post-treatment injury from dry storage is entirely prevented by washing the seed with water immediately after treatment.

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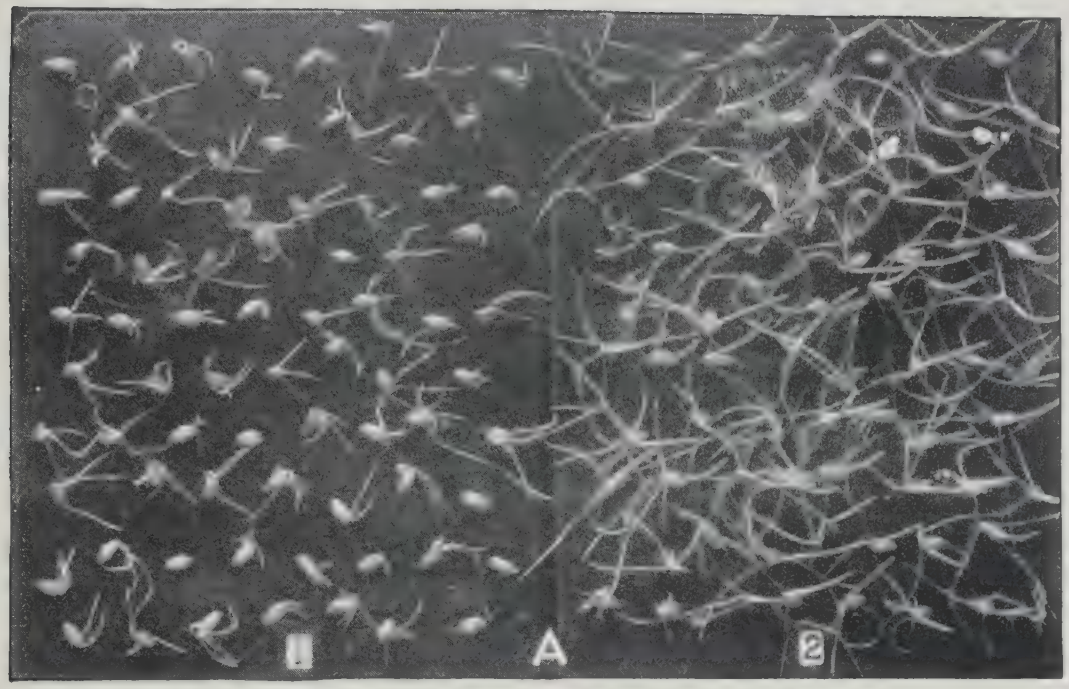
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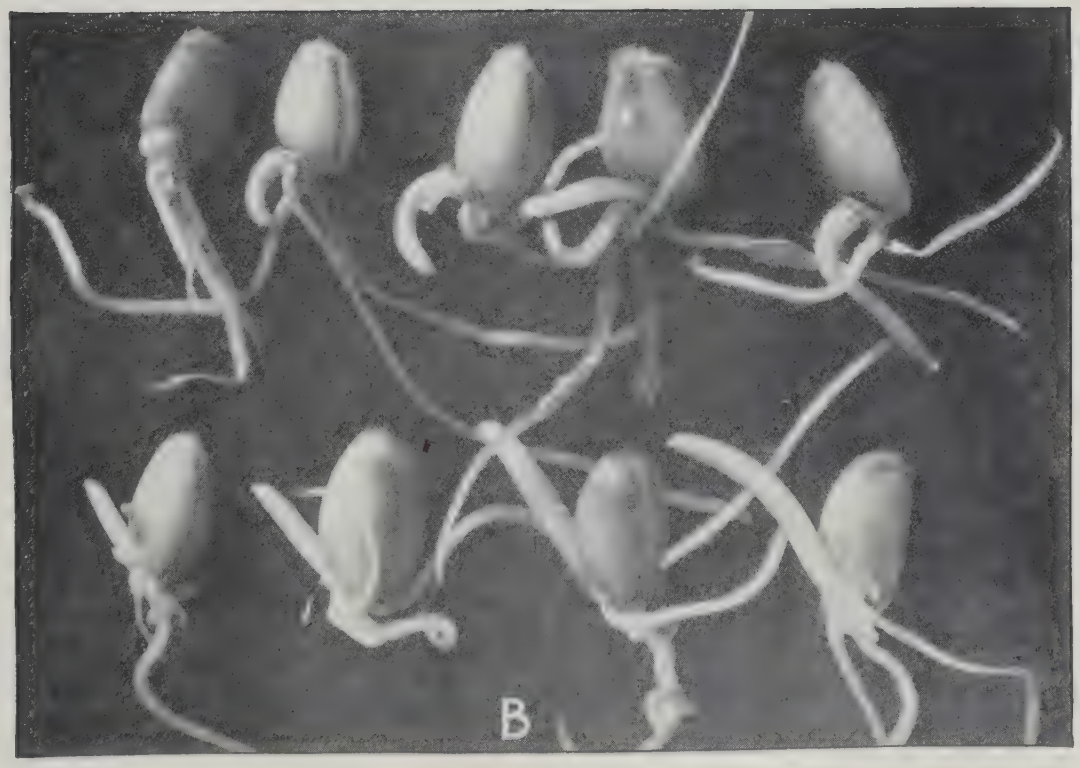
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PLATE 36

A.—Post-treatment seed injury occurring when wheat is dried after treatment with a 0.1 per cent solution. Sample No. 1 was stored dry during the 28 days preceding this germination test, and sample No. 2 was stored damp in a sealed jar, the latter germinating at the end of that time as well as the untreated control.

B.—Germinating seedlings of Little Club wheat, showing characteristic post-treatment injury when seed is treated with a 0.1 per cent solution. The upper row shows the usual deformity—curved, sickle-shaped plumule and prematurely broken sheath. Below are seedlings from untreated seed, showing normal germination.


1
A
2


B


2
4
6
7
1
3
5
A


1
2
3
4
5
B

PLATE 37

A.—Pots showing germination of treated seed stored for 32 days after disinfection with a 0.1 per cent solution of formaldehyde: No. 1, stored dry in laboratory; No. 2, stored damp in laboratory; No. 3, stored dry in refrigerator; No. 4, stored damp in refrigerator; No. 5, stored dry in greenhouse; No. 6, stored damp in greenhouse; No. 7, control, untreated.

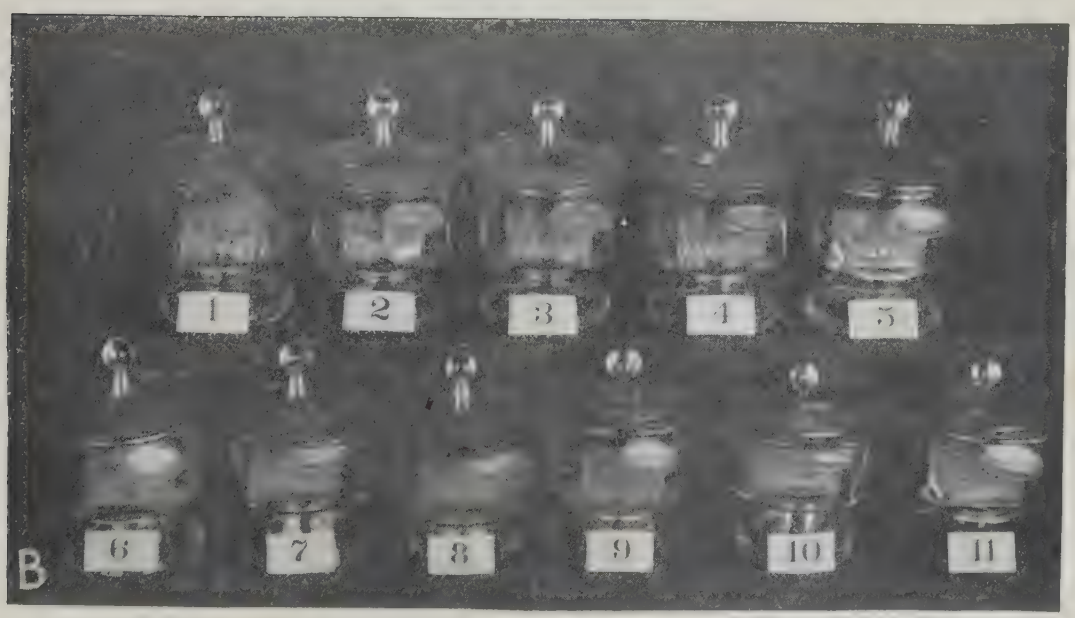
B.—Wheat plants grown in soil from seed stored for 60 days after disinfection with a 0.1 per cent solution of formaldehyde: No. 1, stored dry in refrigerator, germination 18 per cent; No. 2, stored dry in laboratory, germination 34 per cent; No. 3, stored dry in greenhouse, germination 70 per cent; No. 4, stored damp in greenhouse, germination 100 per cent; No. 5, control, untreated, germination 100 per cent.

PLATE 38

A.—Wheat seedlings showing injury produced by allowing the seed to lie in dry soil for 30 days after treatment with a 0.1 per cent solution of formaldehyde: Left, control, dipped in water, 100 per cent germination; center, dipped in 1 to 320 (0.1 per cent) formaldehyde, 62 per cent germination; right, dipped in 1 to 160 (0.2 per cent) formaldehyde, 48 per cent germination.

B.—Desiccators with different degrees of atmospheric humidity obtained by the use of mixtures of sulphuric acid and water in different proportions. The dishes containing formaldehyde were not placed in the desiccators until after the degree of injury to the treated seeds had been determined. The atmospheric humidities were as follows: No. 1, saturated; No. 2, 90 per cent; No. 3, 80 per cent; No. 4, 70 per cent; No. 5, 60 per cent; No. 6, 50 per cent; No. 7, 40 per cent; No. 8, 30 per cent; No. 9, 20 per cent; No. 10, 10 per cent; while No. 11 was absolutely dry, over undiluted acid. Note the white paraformaldehyde formed in these dishes in the drier chambers, beginning with No. 5. (See Table VII for specific gravity readings of sulphuric acid and water mixtures.)


A


1
2
3
4
5
6
7
8
9
10
11
B

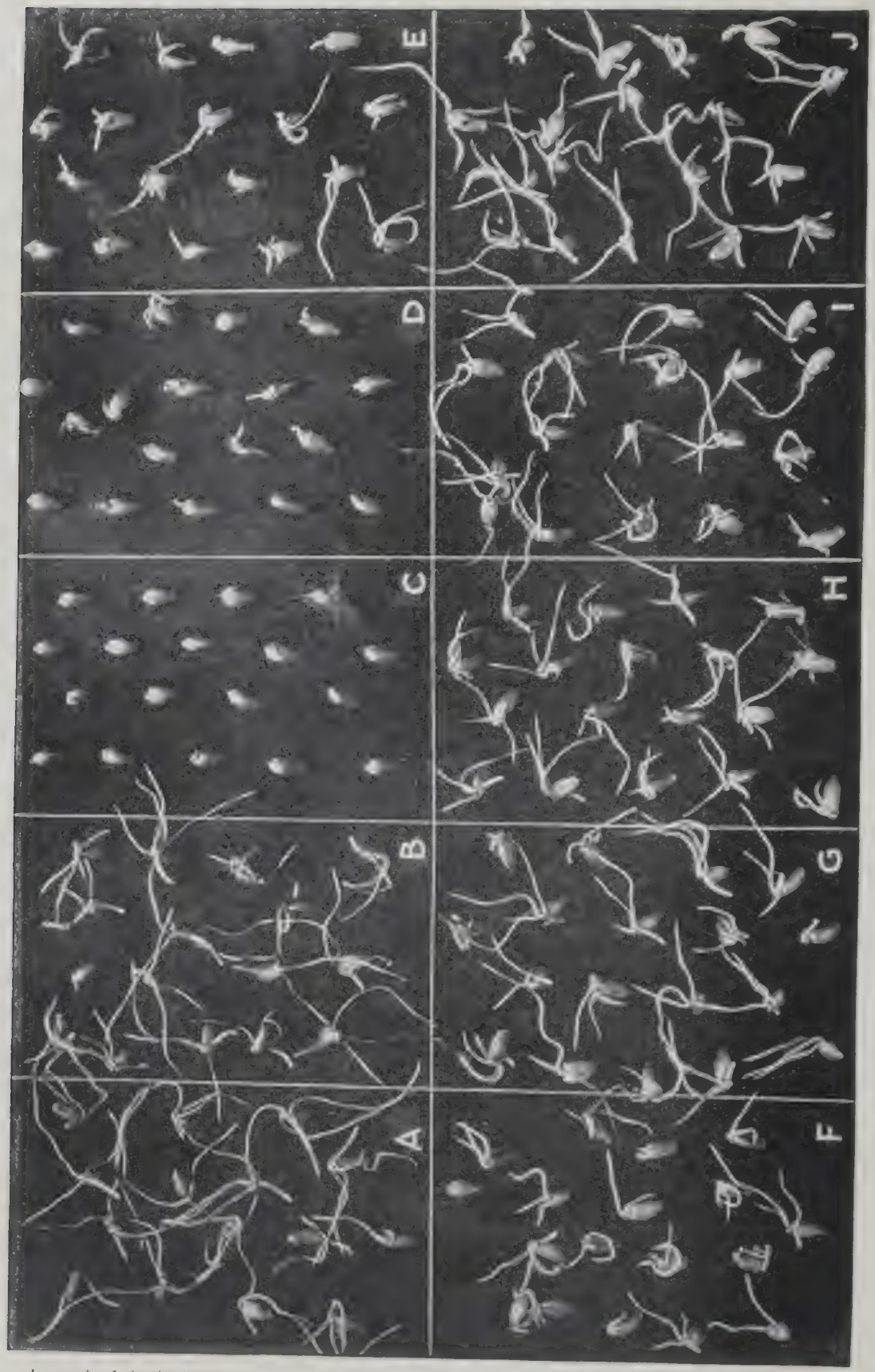

A
B
C
D
E
F
G
H
I
J

PLATE 39

Germinating samples of wheat stored for 35 days after treatment in the desiccators shown in Plate 38 B, illustrating the relation of seed injury to humidity.

Sample A, 100 per cent humidity, 95 per cent germination.
Sample B, 80 per cent humidity, 100 per cent germination.
Sample C, 70 per cent humidity, 0 per cent germination.
Sample D, 60 per cent humidity, 20 per cent germination.
Sample E, 50 per cent humidity, 45 per cent germination.
Sample F, 40 per cent humidity, 80 per cent germination.
Sample G, 20 per cent humidity, 90 per cent germination.
Sample H, 10 per cent humidity, 80 per cent germination.
Sample I, 0 per cent humidity, 100 per cent germination.
Sample J, control, untreated, 100 per cent germination.

PLATE 40

Varying injury to wheat treated with a 0.1 per cent solution of formaldehyde, and stored in sealed bottles:

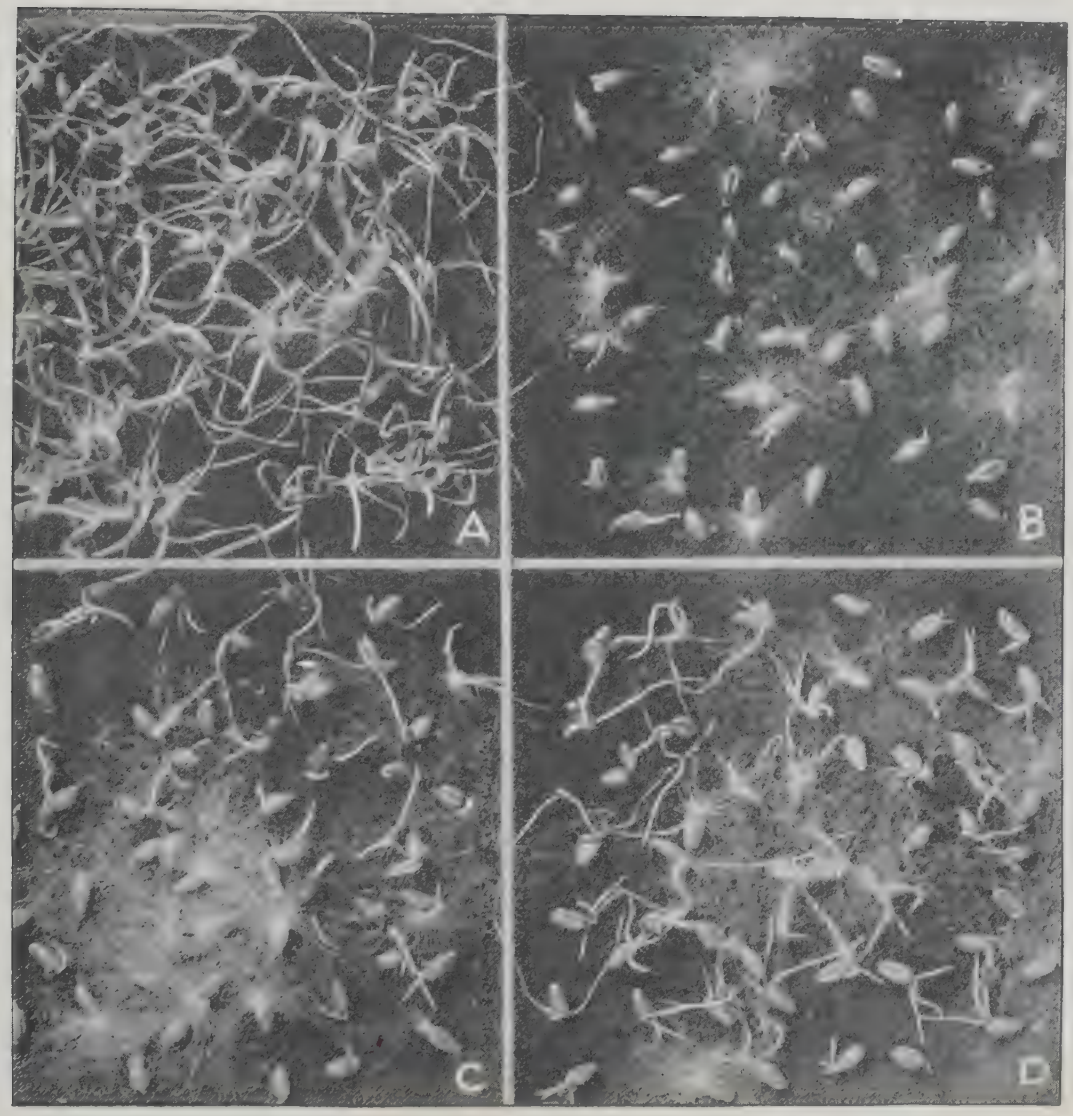
A.—Sealed immediately after treatment, 100 per cent germination.

B.—Sealed after drying 7 hours, spread on towels in laboratory, no germination.

C.—Sealed after drying 24 hours, spread on towels in laboratory, no germination.

D.—Sealed after drying 3 days, spread on towels in laboratory. 14 per cent germination.

The control germinated 96 per cent.


A
B
C
D

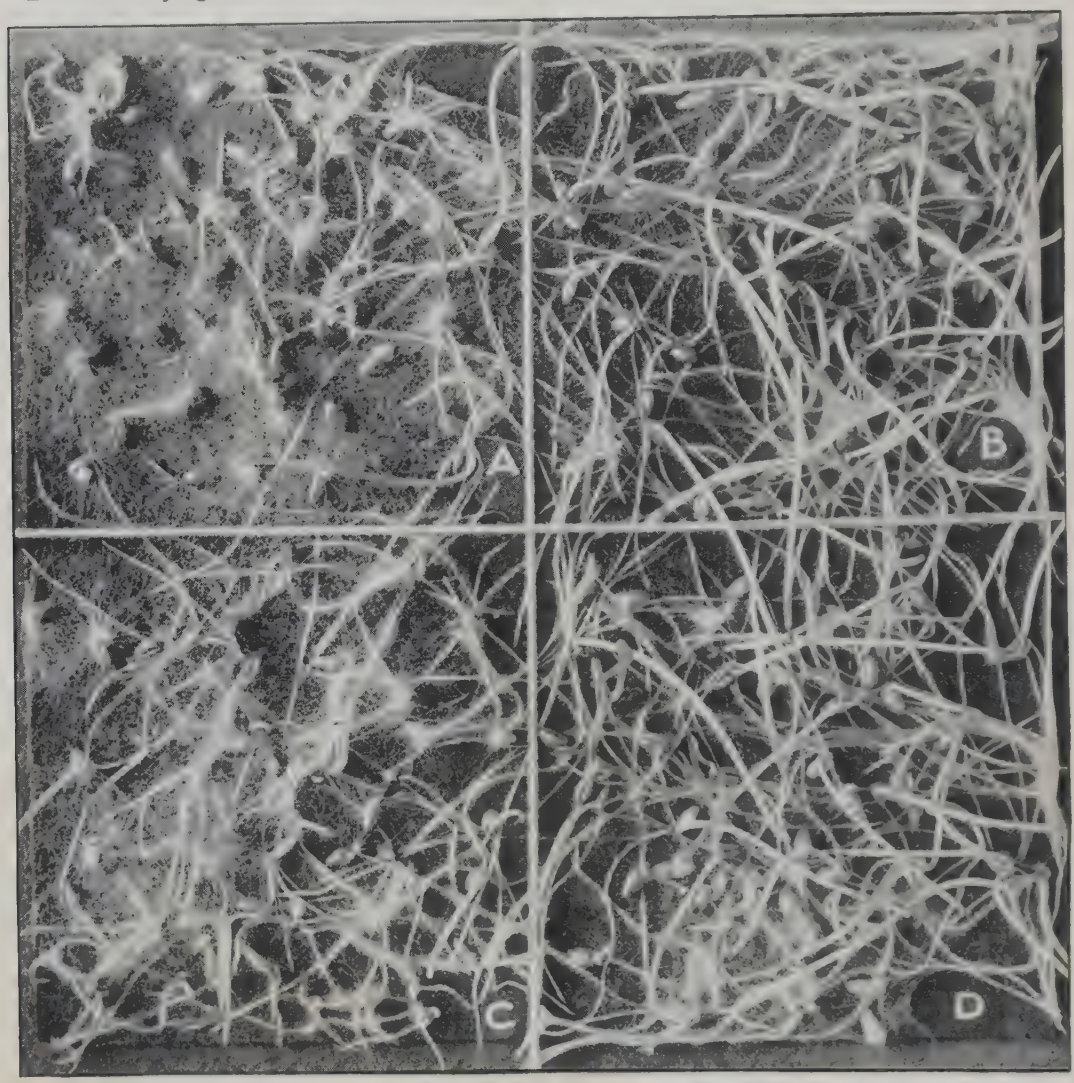

A
B
C
D

PLATE 41

Germinating wheat kernels, showing the prevention of post-treatment injury by washing the seed with water immediately after treatment. Susceptibility of seeds injured by treatment to Rhizopus and other saprophytes is also shown. This seed had been kept in open tumblers for 30 days after treatment.

A.—Treated with 0.2 per cent solution, which was not washed off before drying, 32 per cent germination.

B.—Treated with 0.2 per cent solution, which was washed off before drying, 76 per cent germination.

C.—Treated with 0.1 per cent solution, which was not washed off before drying, 52 per cent germination.

D.—Treated with 0.1 per cent solution, which was washed off before drying, 74 per cent germination.

Control germinated 74 per cent.

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STUDIES ON THE LIFE HISTORY AND HABITS OF THE BEET LEAFHOPPER[1]

[PRELIMINARY PAPER]

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INTRODUCTION

Much has been published concerning the distribution and history of the beet leafhopper and its relation to the curly-top disease of sugar beets, but no complete account of its life history and habits has appeared. The present paper gives a brief summary of observations bearing on these points made during the past few years at Jerome, Idaho, and in the sugar-beet growing regions of California.

DESCRIPTION

EGG

The egg when first laid is transparent, elongate, and slightly curved. The posterior end tapers gradually almost to a point. Length 0.0612 to 0.0696 mm.; average width 0.0182 mm.

As the embryo develops, faint spots which later become conspicuous eye spots appear on either side of the anterior end. During development the color of the egg changes from white to lemon yellow with a slight tinge of green.

NYMPH

The recently hatched nymph is nearly transparent, with a light yellow tinge in the thorax and abdomen. The antennæ are hairlike and more than half as long as the body. The head is wider than the thorax or abdomen and is the most distinctive characteristic of this instar.

After the first molt the nymph is more slender and the head and antennæ are not nearly so conspicuous. Average length 1.40 mm.; width 0.45 mm. Color usually milky white with a green tinge. Faint brown blotches may be distinguished on the thorax.

In the third instar there is more variation in the coloring. General color varying from yellow with light brown markings to almost black. The pattern made by the brown blotches does not seem to be constant, but the denser coloration on the thorax has been designated as a "saddle" (*3, p. 21*).[2] Length 1.99 mm.

[1] *Eutettix tenella* Baker, suborder Homoptera, family Jassidae.

[2] Reference is made by number (italic) to "Literature cited," p. 252.

The color variations in the fourth instar are similar to those of the third. A red coloration is often observed. The spines on the legs are more conspicuous than formerly, and the wing pads extend to the dorsal margin of the third abdominal segment. Length 2.30 mm.

After the fourth molt the nymph has a slender appearance and is nearly the size of the adult. The wing pads extend approximately to the dorsal margin of the fourth abdominal segment. Length 3.2 mm.

ADULT

In California, during the summer, adults of this species may be collected showing a gradation in color from light green with no markings to dark gray with numerous markings on the elytra (Pl. 42, A–C). In the fall the percentage of dark forms is much larger, and during the winter it is unusual to find a light form. Some of the winter forms appear almost black.

The following color details are given to show, to some extent, the extreme contrast in coloration:

LIGHT FORM (Pl. 42, A).—Front yellow, with faint, light brown, transverse stripes. Eyes gray, with occasional brown spots. Vertex green and lemon yellow, the yellow predominating. Pronotum green. Scutum deep yellow. Elytra hyaline with light brown venation. No pigment in the elytra. Tergum appearing as dark bands through the folded elytra.

DARK FORM (Pl. 42, B).—Front yellow, with irregular, testaceous, transverse bands. Eyes a mixture of red and brown, red usually predominating. Vertex fulvous, apical portion with a white band cut in center by a narrow dark band. Pronotum olive, except for ivory anterior band with several black spots. Scutellum with two square, black spots at basal angles. Elytra subhyaline, marked with black about as follows: Two large, almost circular spots on corium; apical portion and irregular black blotches on claval region. Nervures dark brown, with dark pigment on each side forming irregular bands.

RESEMBLANCE TO OTHER SPECIES

There should be little difficulty in distinguishing the beet leafhopper from other leafhoppers commonly found on sugar beets in California. Occasionally the darkest forms resemble some species of Agallia in coloration, but even a superficial examination will be sufficient to separate these two genera. These species of Agallia do not have the slender appearance of the beet leafhopper and are much slower in their movements. After a little experience in collecting it is possible to distinguish between the two genera by their movements. *Eutettix tenella* rarely, if ever, feigns death when disturbed; but some of the species of Agallia are almost certain to fall over on their backs and lie for some time as if dead. This habit is often an aid in collecting when the leafhoppers are not abundant and a careful search is necessary. One species, *Cicadula 6-notata* Fallen, may often be confused with the beet leafhopper, especially when individuals of the latter are mainly of the green coloration. The six spots on the vertex of *C. 6-notata* are usually plainly evident, however, and will serve to distinguish this species from *E. tenella*.

LIFE HISTORY AND HABITS

REPRODUCTION

During the summer season mating occurs within a few days after the last molt is accomplished, but during the fall this period is greatly prolonged. In Idaho adults were observed copulating in cages during the late fall as well as during the summer season. At Spreckels, Calif., mating continued throughout the winter. Unfertilized females have been known to lay sterile eggs under certain conditions, but parthenogenesis has never been observed.

The preoviposition period is comparatively long. In all experiments 15 to 17 days elapsed between the date the female reached maturity and the date the first eggs were laid. A much longer period is common, especially during the winter and early spring.

OVIPOSITION

Under normal conditions the eggs of the beet leafhopper are usually placed in the petiole or midrib of the sugar-beet leaf, beneath the fibrous strands and at a slight angle. They are invariably deposited one at a time, but often they are arranged in rows of from two to five, placed end to end so that they give the appearance of overlapping. It is almost impossible to find the recently deposited eggs in the petioles; but after the embryo has developed a little and the eye spots have appeared they are comparatively conspicuous. When deposited in the leaf tissue the eggs are more easily detected by the raised areas on the leaf surface. By transmitted light eggs in this position appear as small, transparent slits.

While apparently preferring the sugar beet as a plant in which to deposit its eggs, this leafhopper will oviposit in a large number of other plants. Fleshy or succulent species offer the most suitable conditions for oviposition. Russian thistle (*Salsola kali* var. *tenuifolia*), filaree (*Erodium cicutarium* and *E. moschatum*), *Chenopodium* spp. (especially *murale*), and *Atriplex* spp. are plants from which eggs have been most commonly noted hatching under natural conditions. Most perennial plants are too tough and woody to be suitable for this purpose, and it is doubtful if they are of any great importance as hosts during the egg-laying period.

Ball (*2, p. 40*) records the number of eggs deposited by a single female of this species as about 80. At Spreckels, Calif., the maximum number of eggs deposited by one female was 237, while at Riverside, Calif., the maximum was 247. Many difficulties were encountered in the conduct of these experiments, and it is probable that, given more favorable conditions, the females might have deposited a larger number of eggs.

Meteorological conditions influence greatly the incubation period. A maximum period of 52 days has been observed during the early spring and a minimum of 10 under most favorable conditions. During the

height of the egg-laying season the incubation period ranged from 10 to 15 days.

Seasonal variations in the development of the nymph are wide, due mainly to differences in temperature and food supply. The entire nymphal period ranged from 25 to 52 days, while from 4 to 10 days were required for the completion of each instar.

NUMBER OF GENERATIONS

Ball (*1, p. 93; 3*) states that the beet leafhopper is a single-brooded species and implies that such is the case for conditions even as far south as Glendale, Ariz. Experiments conducted at Spreckels, Calif., demonstrated that there were unquestionably at least two generations annually in that locality. Under conditions more favorable than was usual for this part of the Salinas Valley, a third and even a fourth brood were obtained. There was only one brood on sugar beets in southern Idaho, but it seems probable that further investigation would reveal an additional brood, possibly on the wild vegetation.

LONGEVITY OF ADULTS

Under natural conditions it is doubtful if the normal length of life of the adult is more than 4 or 5 months. Fall-brood adults are not found in the fields during the summer, and the spring brood is rarely noted in the fall. Females have been kept alive in cages for 19 months, but it is doubtful if they would ever survive so long under field conditions.

SEASONAL HISTORY

IN SOUTHERN IDAHO

Although persistent effort was made to locate adults of the beet leafhopper during the winter and early spring in southern Idaho, they were not observed until their appearance on the sugar beets. The earliest record for this was June 6, 1914, when several individuals were collected on volunteer sugar-beet plants at Jerome. Apparently the leafhoppers are in the cultivated fields as soon as the beets are up.

Oviposition begins in the field as soon as the adults appear. Records have been made as early as June 22, when the beets were still young and had not yet been thinned. June 28 was the earliest hatching record obtained in cage experiments. Starting thus, early in June, oviposition continues throughout the season until late in October.

During 1913 adults were not observed copulating until late in the fall. On October 12, a large number of adults confined in a lantern globe were noted copulating for several days. During the one winter spent by the writer in this district only a few adults placed in cages in the fall survived the winter, and all of these were females. These observations indicate that the females are fertilized in the fall before hibernation and that a large percentage of males perished during the winter.

Weather conditions were severe enough during the winter in this district to necessitate hibernation. All attempts to determine the method of hibernation, however, as well as the places in which it takes place were failures. Adults in cages survived the winter underneath dead beet leaves and in the crown of the plant.

IN CALIFORNIA

Under California conditions adults and nymphs are most abundant in the field during August. At harvest time they are scattered, and no doubt a large number perish. After the beets have all been removed from the fields the leafhoppers seem to be greatly diminished in numbers, although they may be collected from certain weeds growing in the fields and along the irrigating canals. No indications of a general migration have been noted at such times, so it is assumed that the surviving individuals scatter over wild vegetation, selecting that which is most suitable for food and protection. Later they may congregate in certain spots which furnish especially favorable conditions during winter.

There is no true hibernation in the districts of California that have been under observation. Adults have been collected every week in the winter under conditions indicating that they were feeding when captured. Under cage conditions food must be available at all times. As a rule, all individuals kept without food died within 48 hours.

The characteristic dark-colored individuals of the fall brood that leave the beet fields could hardly be confused with the light-colored adults that appear the next spring. A small percentage of the fall-brood adults may remain in or near the beet fields during the winter and be responsible for the early injury in the spring, but it is usually not until the light forms appear in considerable numbers that attention is directed to the damage. The striking difference in coloration between the fall and spring forms suggests at once the possibility of a new brood on wild vegetation before migration into the beet fields. Observations and cage experiments have proved that such a brood occurs.

The time when the leafhoppers first appear in fields in spring in California varies with the seasonal conditions in different localities, being from April 1 to June 1. The condition of wild vegetation in the natural breeding areas is an important factor in determining when migration to the beet field will take place. As long as this vegetation is abundant and succulent it is doubtful if there is any general movement into the cultivated areas.

Oviposition begins as soon as the adults appear in the field and continues throughout the season. There is an overlapping of broods which makes it impossible to determine the exact number under field conditions. Cage experiments, however, have demonstrated that there may be from one to three each year on the beets. Thus the maximum number of broods in one year would be four.

NATURAL ENEMIES

EGG PARASITES

The following three species of egg parasites have been reared from the beet leafhopper and studied to some extent. They are given in the order of their importance.

POLYNEMA EUTETTIXI GIRAULT (*4, p. 18*) (PL. 43, A).—This small brown or black species was first reared from eggs of *Eutettix tenella* at Spreckels, Calif., early in 1915 and has proved to be the most effective parasite of this group in the Salinas Valley. Eggs parasitized by this species are conspicuous in the petioles of the beets because of the black color of the parasite pupæ. Development is rapid, the life cycle from adult to adult covering about 35 days on an average, and there are at least nine generations annually.

ABBELLA SUBFLAVA GIRAULT.—Concerning this parasite W. J. Hartung (*5*) writes as follows:

> Hyper-parasites were bred from parasitized eggs of Eutettix. These were determined by Girault as *Abbella subflava* Girault.

This species[1] was never found among the parasites reared from material collected at Spreckels, Calif., but at Riverside it was reared in about equal numbers with *Polynema eutettixi*.[1] It is a primary parasite, ovipositing readily in eggs of the beet leafhopper. It has also been reared from eggs of *Empoasca* sp.

ANAGRUS GIRAULTI CRAWFORD.—This common orange or red jassid egg parasite has been reared in each locality where parasite studies have been conducted. It oviposits readily in eggs of the beet leafhopper and is usually reared along with *Polynema eutettixi*, but not in such large numbers. The presence of this species in the petioles of the beet can be detected by the red or orange color found in both larva and pupa.

PARASITES OF THE NYMPHS AND ADULTS

As previously reported by Hartung and Severin (*6*), two species of the dipterous family Pipunculidae are known to be parasitic on the nymphs and adults of the beet leafhopper. These have been described (*7*) as *Pipunculus industrius* Knab and *Pipunculus vagabundus* Knab. The former is the more common species in the Salinas Valley.

PIPUNCULUS INDUSTRIUS KNAB (PL. 43, B).—Eggs of this species are deposited in both nymphs and adults of the beet leafhopper, but mature larvæ have never been known to emerge from a nymph. There are no indications that the adult female prefers either the mature or immature stages of the host in which to deposit her eggs, very small parasitic larvæ having been dissected in about equal numbers from both stages. It is known, by dissection, that eggs may be deposited in small nymphs

[1] Specimens identified by Mr. A. B. Gahan.

no further developed than the third instar. In all instances, however, where an action thought to be oviposition was observed, the adult host was the victim.

The adult is very graceful in flight, darting here and there so suddenly that it is impossible to follow the movements with the eye. The beet leafhopper, also, is very quick in its movements, but none is quick enough to avoid this active little parasite.

PIPUNCULUS VAGABUNDUS KNAB.—This species is not common in the Salinas Valley and is of little importance. Its habits are similar to those of *Pipunculus industrius*, and, with the exception of the conspicuous stigma which is absent in the wings of *P. vagabundus*, the two species are similar in appearance.

DRYINIDAE.—Occasionally beet leafhoppers, both adults and nymphs, are found with a dark brown sac or pouch protruding from the abdomen (Pl. 42, D). This pouch contains the larva of a dryinid parasite. Hartung and Severin (*6*) report a parasite of this family, *Gonatopus contortulus* Patton, from the Salinas Valley. Although the writer has reared many specimens of this family, none has been determined. Judging from the number of parasitized leafhoppers collected, these dryinids are not of much economic importance. It has been observed, however, that the adults devour a larger number of the leafhoppers, especially nymphs, than they parasitize. In this way they may be of more importance than would at first appear.

SUMMARY

Eggs of *Eutettix tenella* are deposited in a wide range of cultivated and wild plants, but the sugar beet seems to be preferred for this purpose during the summer season. A maximum record of 247 eggs was obtained for a single female. The incubation period covered from 10 to 15 days during the height of the egg-laying season and the nymphal period from 25 to 52 days.

One generation only was observed in southern Idaho, while from two to four were observed under California conditions.

In southern Idaho the beet leafhopper appears in the beet fields in June and starts reproducing at once, oviposition continuing throughout the season. After harvest the leafhoppers enter a true hibernation period.

In California the adults appear in the beet fields soon after April 1 and remain until harvest time, when they disperse to wild vegetation suitable for food and protection. No true hibernation was noted in California.

Three species of egg parasites were reared and studied. Two of these are very effective. Two species of Pipunculus, internal parasites of the nymphs and adults, were reared; and one of these was quite effective. Dryinid parasites, also, were reared but are not considered very efficient.

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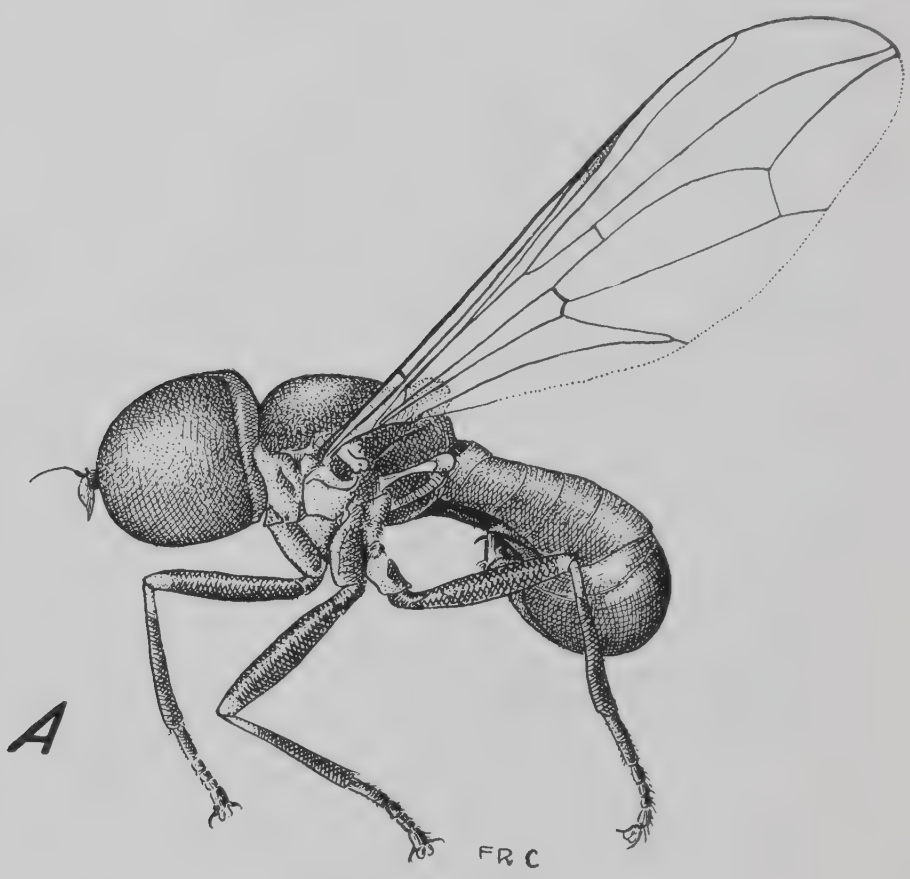
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PLATE 42

Eutettix tenella:

A.—Adult, light form.
B.—Adult, dark form.
C.—Adult, color gradation between A and B.
D.—Nymph with protruding sac of dryinid parasite.
All much enlarged.


A
B
C
D


A
FRC

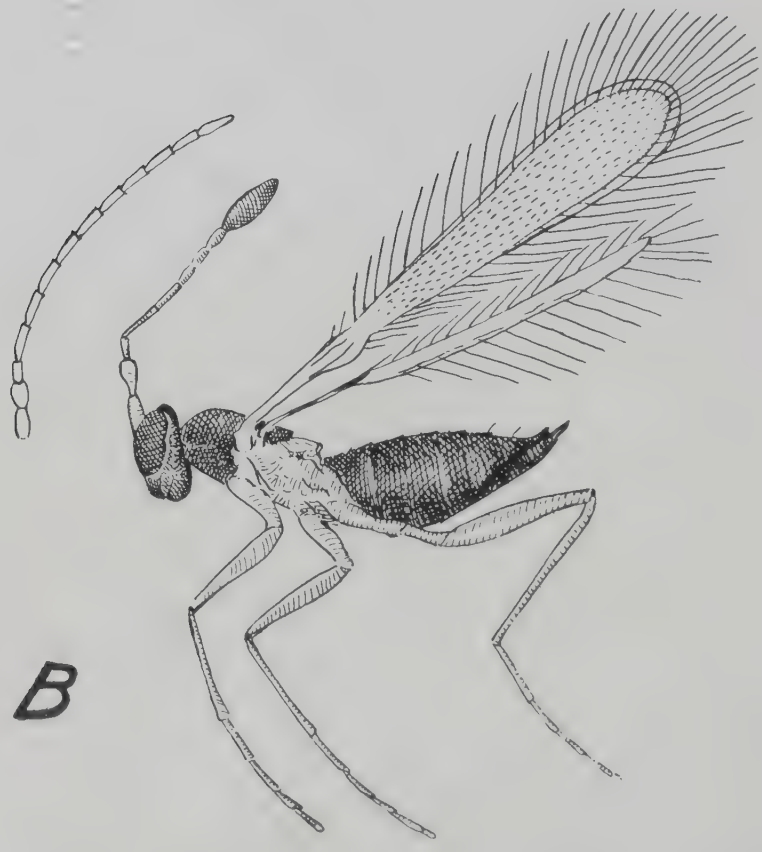

B

PLATE 43

Parasites of *Eutettix tenella:*

A.—*Pipunculus industrius:* Adult, much enlarged.
B.—*Polynema eutettixi:* Adult, much enlarged.

HYPERTROPHIED LENTICELS ON THE ROOTS OF CONIFERS AND THEIR RELATION TO MOISTURE AND AERATION

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INTRODUCTION

At the Bessey Nursery of the United States Forest Service at Halsey, Nebr., warty excrescences were observed upon the roots of coniferous seedling stock during the shipping season of 1915. Such excrescences occurred on all pine species grown there. They were so abundant on western yellow pine (*Pinus ponderosa*)[2] that the possibility of a parasite as the causal agent was suggested, and the forest officers properly questioned the advisability of shipping the stock to other regions.

Attempts were made by the writers to obtain evidence of a pathogenic organism, but always with negative results. This experimentation consisted of (a) incubation in moist chambers of portions of roots bearing excrescences, (b) insertion of the interior portion of the excrescences, removed with aseptic precautions, into nutrient agar, and (c) inoculation of portions of the excrescences into roots of healthy 2-year-old and 4-year-old *Pinus ponderosa* stock.

After the failure to obtain evidence of a pathologic organism, a histological examination was made, which showed that the excrescences had the structure of the hypertrophied lenticels (Pl. 44) so commonly seen in many dicotyledonous plants.

DESCRIPTION

The hypertrophied lenticles are found both upon the main tap root (Pl. 45, B) and upon the lateral roots, not only close to the ground level and upon the stems proper but also on the tap roots as far as 14 inches (36 cm.) below the surface of the soil.[3] On the stems of conifers the hypertrophied lenticles usually occur only on the basal portions of trees growing in abnormally wet situations (Pl. 45, A) or on parts otherwise submerged. In exceptionally humid situations they may occur occasionally on parts of the stems above the soil surface.

[1] The writers wish to acknowledge helpful suggestions from Dr. B. E. Livingston, of the Johns Hopkins University, and Dr. T. H. Goodspeed, of the University of California.

[2] All the western yellow pine referred to in this paper was the type sometimes referred to as *Pinus ponderosa* var. *scopulorum*, from eastern Rocky Mountain seed.

[3] In all probability hypertrophied lenticels will be found at much greater soil depths on the roots of older trees.

On the small roots the hypertrophied lenticels occur most commonly, but not always, at the junction of a lateral root or rootlet with its parent root, usually originating immediately above the point of origin but also subtending, at the sides or immediately below, the root or rootlet in question. This agrees with the findings of De Vaux (*5*)[1] on normal lenticels, who reports that primary lenticels on roots are always at the bases of root branches, though secondary lenticels are sometimes formed later at other points. It was this coincidence of lenticels and root branches that caused some botanists during the early part of the nineteenth century to believe lenticels equivalent to buds, a doctrine attributed to De Candolle (*7; 13, Vorwort*) and overthrown by Majer (*13*),[2] Unger (*22*), Terras (*19*), and others.

The excrescences vary greatly in size and shape, from minute circular areas 0.5 mm. in diameter to bands nearly encircling the larger roots in cases where two or more lenticels have become laterally confluent. Around the root crowns and the bases of the submerged stems large, wartlike patches may occur, 5 to 8 mm. in diameter and projecting 1 to 3 mm. above the surface of the bark. Examination with a dissecting microscope shows these excrescences to be made up of a very loosely piled mound of pale yellowish tissue. As a general rule these mounds of loosely piled cells split in a stellate manner, the segments recurving outward, occasionally leaving a few filamentous columns standing by themselves in the center. Such structure is evident only when the young trees have been removed from the ground with great care, for the slightest touch upon these loose-lying columns causes them to crumble instantly to a flat, powdery mass, especially when they are dry. On the bases of still older stems 1 to 2 inches (2.5 to 5 cm.) in diameter that stand for a large part of the growing season in water or poorly drained soil, the bark, which is here considerably thickened, exfoliates in patches of varying size, revealing irregularly connected flattened masses of cells, or, more rarely, unbroken areas of such cells 1 inch (2.5 cm.) broad. On some pines these excrescences frequently become so abundant that considerable areas of the lower stem and the tap root are covered by them (Pl. 46, B). After the cessation of growth in the lenticels, these excrescences become dark root-brown and gradually slough off.

The lenticellular excrescences vary in different conifers from loosely connected, more or less divergent, columnar masses crumbling at the slightest touch, common in the pines, to fairly compact, corky masses usually seen in the trees of other coniferous genera.

Histological examination of the excrescences at once proves the white, spongy tissue to consist of more or less loosely connected masses of cells developed from the phellogen. Plate 44 illustrates a cross section of

[1] Reference is made by number (italic) to "Literature cited," p. 264–265.

[2] This seems to be the 1836 paper attributed to Mohl by Haberland (*7*). Mohl apparently directed the work of Majer and wrote a preface for the dissertation, but Majer was the author of the paper itself.

one of these hypertrophied lenticels on a root of *Pinus rigida*. The outgrowths consist of homogeneous parenchymatous elements, more or less radially elongated, sometimes very much so. The individual cells are thin-walled with a thin layer of cytoplasm.

SPECIES AFFECTED

Stahl (*18*) states that all trees which have lenticels on the stems also have them on the roots. De Vaux (*5*) reports the presence of lenticels on the roots of a large number of tree species, including a number of conifers. For one species of Ephedra he states that lenticels are found only on the roots. He states that especially in *Pinus maritima* the lenticels on the roots are larger than those on the stems. This author was able to find or to produce lenticel hypertrophy on some part of the plant in 60 per cent of the 155 plant species considered but was unable to secure any hypertrophy on the representatives of the several coniferous genera which he studied. On roots less than 3 mm. in diameter he found the normal lenticels so small that the microscope was usually necessary in demonstrating them. Tubeuf (*20*) lists a small number of species, of which he was able to secure lenticel hypertrophy on some part of 12 nonconifers. He, however, failed to get this hypertrophy on species of Sequoia, Thuja, and Taxus, or on *Gingko biloba* and 14 other nonconiferous species. Zach (*23*) later secured hypertrophy of lenticels on stems of *G. biloba* under certain conditions. However, a rather careful search in the earlier literature appears to justify the statement by the reviewer of Zack's paper (*16*) that no hypertrophy of lenticels had been up to that time reported on conifers.

The present writers have found hypertrophied lenticels on the roots of the following conifers: *Pinus ponderosa, Pinus coulteri, Pinus rigida, Pinus resinosa, Pinus banksiana, Pinus virginiana, Pinus sylvestris, Pinus caribaea, Pinus strobus, Pinus monticola, Pinus excelsa, Picea canadensis, Picea rubens, Picea mariana,*[1] *Picea pungens, Abies balsamea,*[2] *Tsuga canadensis, Larix laricina, Taxus cuspidata, Taxus brevifolia*, and *Araucaria bidwellii*.

Several of the species of Pinus on which the hypertrophy was found were growing in the greenhouse of the United States Department of Agriculture at Washington, D. C. It was noteworthy that plants of *Juniperus virginiana* under the same conditions in the same greenhouse apparently were free from such growths so far as could be determined. In a swamp in which the hypertrophied lenticels were found on *Abies balsamea, Picea rubens*, and *Tsuga canadensis* none could be discovered on *Taxus canadensis*. Among the pines the hypertrophied lenticels were frequent mainly on the 3-needled species, *Pinus ponderosa* and *Pinus*

[1] Material furnished by Dr. H. P. Brown, of The New York State College of Forestry at Syracuse University.

[2] Dr. James R. Weir advises the writers that he has frequently found hypertrophied lenticels on the roots of *Abies grandis* in the Northwest.

rigida, while on the strictly 2-needled *Pinus virginiana*, *Pinus banksiana*, and *Pinus resinosa* they were very difficult to find. Klebahn (*10*, *p.* 582, 586) states that up to the time of his publication he had not been able to find lenticels on *Pinus sylvestris*, nor had he satisfactorily demonstrated a substitution for lenticels.

Excrescences like those just described on the conifers are common and widespread occurrence on a number of dicotyledonous plants, particularly upon swamp plants such as *Sambucus canadensis*, *Rhus copallina*, *Decodon verticillatus*, and *Cephalanthus occidentalis*. Such excrescences on dicotyledonous plants have long been known under the term "water lenticels."

CONDITIONS UNDER WHICH HYPERTROPHY HAS BEEN FOUND

The lenticel hypertrophy observed on roots has been generally limited to plants growing in wet soil. Affected hemlock, balsam fir, red spruce, and black spruce have already been noted as growing under swamp conditions. All the pitch pines found with hypertrophied lenticels in the vicinity of Washington were in heavy, wet soil. There hypertrophy was very frequent on *Pinus rigida* and *P. virginiana* growing in swampy locations. The pines found so affected in the greenhouse at Washington were all growing in soil very much wetter than that in which they are usually found. The only Scotch pines found with hypertrophied lenticels were growing at the edge of an irrigation ditch in especially wet soil at a Michigan nursery. The same has been true in the most striking cases of hypertrophy at the Bessey Nursery. In a bed, a portion of which was repeatedly flooded from a leaking irrigation ditch, approximately 20 per cent of the plants showed marked cases of hypertrophy, while less than 1½ per cent of the plants showed hypertrophy in parts of the neighboring beds which were not affected by the leakage. Information has been received from Mr. W. H. Schrader that at the Monument Nursery of the United States Forest Service in Colorado the only conspicuous occurrence of root lenticel hypertrophy was during an unusually wet season. The hypertrophy here considered has been found both in heavy and in very sandy soils; in the latter case there was apparently more hypertrophy in parts of the beds to which clay had been added.

The youngest seedling observed with lenticel hypertrophy was one of *Pinus ponderosa* which was raised from the seed with its roots in a 2-ounce bottle of tap water in the laboratory. This water was not changed during the entire period of growth. The bottle was stoppered but was not absolutely sealed at the point of passage of the stem through the stopper. At the end of approximately five months the plant, which still seemed fairly vigorous, had developed a single root, which, after reaching the bottom of the bottle, had coiled itself around two or three times close to the peripheral limit of the bottle. On this tap root were a

number of conspicuous, glistening, mound-shaped excrescences, as is shown, slightly magnified, in Plate 46, C. A microscopic examination of sectional preparations of these excrescences (Pl. 46, A) showed clearly their lenticellular structure. The outgrowths were so loose and delicate that the outer portions were necessarily lost in sectioning, but the figure shows enough of the bases to indicate the type of structure.

In general, root-lenticel hypertrophy has been found especially frequent not only on species like western yellow pine, which are somewhat inclined to lack fine fiberous roots, but also on individuals of other species when a strong tap root has been developed with relatively little development of laterals. Whether or not the larger lenticels are of advantage to such plants in fulfilling part of the functions that the missing laterals might have performed is of course uncertain. In this connection it is of some interest to note the finding of root-lenticel hypertrophy in Michigan on white and Colorado blue spruce (*Picea canadensis* and *P. pungens*) whose roots had been injured by May beetle larvae. It is also especially interesting that nursery trees that have not been transplanted or that are in their second season in the transplant beds show decidedly less hypertrophy than recently transplanted stock. The recently transplanted trees have, of course, lost most of their absorbing roots, while the trees transplanted the preceding season have had a chance to develop normal root system again after transplanting.

IRRIGATION EXPERIMENTS

Trees of *Pinus ponderosa* in their third year in the nursery, and two months following transplanting, were given river water from the irrigating ditch frequently during a three months' period, beginning July 11, 1917. All the tests considered in this and the following section were conducted at the Bessey Nursery in cooperation with Forest Supervisor Jay Higgins and his assistants. The water added at each irrigation was approximately equivalent to 2.2 inches (5.6 cm.) of rainfall. A bed which received 31 such irrigations during these three months showed at the end of the period 31 per cent of the trees with 8 or more distinctly hypertrophied lenticels each and a total of 57 per cent with some evidence of hypertrophy. The figures are based on an examination of 255 trees. This amount of watering was sufficient to cause more or less chlorosis, especially of the shoots which arose after the watering began. Another bed in the same section, on which the frequent watering was not started until a month later and which received during the entire three months a total of 17 irrigations, showed at the end of the period eight or more enlarged lenticels each on approximately 13 per cent of the plants examined. Other beds used as controls received the usual amount of water given at this nursery, involving six irrigations in addition to the 7.7 inches (20 cm.) rainfall during the period of three months.

These showed less than 1½ per cent of the plants with abundant hypertrophied lenticels and a total of less than 13 per cent showing any evidence of hypertrophy. The results in the most heavily watered bed and in the controls are given in Table I. The results with the pruned trees shown in the table lead to the same conclusions as the results cited above on the unpruned trees—namely, that heavy watering increased the amount of lenticel hypertrophy.

TABLE I.—*Effect of watering and top pruning on root-lenticel hypertrophy of third-year western yellow pine at Bessey Nursery, Halsey, Nebr., pruned in early July and examined September 10 to 15*

Plot.	Part removed by pruning.	Number of trees examined.		Percentage of trees with hypertrophy.		Percentage of trees with strong hypertrophy.[a]	
		Heavily watered series.	Normally watered series.	Heavily watered series.	Normally watered series.	Heavily watered series.	Normally watered series.
C	All the secondary needles [b]	185	42	8	7	3	0
B	All the secondary needles [b] and tip of third season terminal shoot.	182	47	9	2	2	0
A	All the secondary needles [b] and entire third-season shoot	32	51	6	0	0	0
E	Third season terminal shoot only	108	48	41	17	19	0
D	Half the secondary needles only [b]	58	0	31	.....	17	.....
F	Unpruned	206	72	58	11	33	0
.....	Additional unpruned rows scattered among the different series	49	71	51	13	24	3
ABC	Heavily pruned	399	140	9	2.9	2.3	0
DE	Lightly pruned	166	48	37	17	18	0
.....	Unpruned	255	143	57	12	31	1.4

[a] Having 8 or more noticeably hypertrophied root lenticels per tree.
[b] Including the needles that had appeared on the third-season shoot as well as those produced in earlier years. Cut back to sheath but portion of needle remaining in the sheath left intact.

PRUNING EXPERIMENTS

Pruning experiments were conducted in an effort to throw a little more light on the factors controlling the lenticel hypertrophy. The tops of a number of rows of western yellow pine transplants at the Bessey Nursery were pruned with different degrees of severity during the first week in July, 1917. This is about the middle of the season of vigorous growth at this nursery. The results of a root examination three months later appear in Table I. The most heavily pruned plants showed the least lenticel hypertrophy, with the exception of plot E in the normally watered series. The percentage in this case is based on only 48 trees, only one-third as many as furnished the basis for each of the other figures in the three lower lines of the table. The pruning did not so injure the plants as to prevent growth entirely, for even those most heavily pruned reacted by sending out new shoots.

CAUSES OF LENTICEL HYPERTROPHY

Schenck (*15*) attributed lenticel growth on roots to oxygen hunger. However, the association which has been observed between moist conditions and abnormal lenticel growths, as well as experience in artifically producing lenticel hypertrophy by placing cuttings in water or moist air, have led more recent writers to suppose that for dicotyledonous plants the hypertrophies are directly due to the presence of an unusual amount of water (*5; 11, p. 72–80; 17*). It is reasoned, in the first place, that water or constantly moist atmosphere on the outside of the lenticels allows the steady growth of the lenticels, while dry or intermittently dry air tends to dry out the superficial cells of the lenticels or to increase their solute concentration, with resultant chemical changes, including cork and lignin formation. According to this idea the growth of the lenticel tissue is controlled by transpiration through the lenticels; with intense transpiration the tissues become dried and the hypertrophy is checked. The suberized or lignified layers thus formed are supposed to restrain mechanically further proliferation on the part of the cells beneath them from which the lenticel structures arise. So far this supposition seems logical, though there is as yet no basis for a quantitative estimation of the importance of tissue drying in the phenomenon.

DeVaux has advanced another theory, based on the fact that the supplying of abundant water to the absorbing surfaces and the reduction of transpiration have both been found to be followed by lenticel hypertrophy in experiments with dicotyledons. This writer supposes that both these treatments result in increased sap pressure in the plant as a whole and exert their influence entirely through increased sap pressure. He does not apparently give sufficient weight to the possibility that both decrease in transpiring surface and increase in soil moisture may involve decreased oxygen supply as well as increased sap pressure. The limited aeration of wet soils is a matter on which there is general agreement. The necessity of soil oxygen for the normal development of mesophytic plants, as indicated by common observation, has been recently confirmed by direct experiments by Cannon and Free (*3*) and by Livingston and Free (*12*). It is by no means certain that over-wet soil results in increased sap pressure in mesophytic plants, especially since the last-named authors find that a deficiency of oxygen in the soil results in some cases in decreased water absorption. The association between swampy soil and lenticel hypertrophy is at least as easily explained on the basis of oxygen hunger as by DeVaux's "hyperhydrose" doctrine.

The argument which Tubeuf (*20, 21*) seems to consider strongest against oxygen hunger as the stimulus for lentical enlargement is the fact that enlargement can be produced in cuttings in a moist chamber. By placing cuttings with paraffined ends in moist chambers he secured lenticel overgrowth, even in cases in which an atmosphere of oxygen was

provided. This seems at first glance to dispose of the oxygen-hunger hypothesis quite effectively. However, an atmosphere of oxygen would not necessarily insure an oxygen supply to the interior of a woody stem unless the lenticels were already open at the time the cutting was placed in the chamber. A section of stem removed from the plant and therefore deprived of the oxygen that it would normally get from the leaves and perhaps also from the roots, if its lenticels were closed, might easily by oxidation of stored food materials develop abnormal partial pressures of carbon dioxid in its interior tissues which would not be relieved till the lenticels were opened by the stimulated growth which Tubeuf describes. The experience reported in his later paper, in which he records interesting cases of lenticel stimulation secured by covering bark with impervious materials, and observation of lenticel hypertrophy on the swelling above a heat lesion lead him to consider the stimulation lenticel growth too complicated to be explained by any single factor so simple as humidity. He still appears to consider oxygen hunger as excluded from further consideration. However, his observation of numerous lenticels on the stem of a heart-rotted spruce is the only reference that has been found concerning abnormal lenticel growth on any part of a coniferous tree.

The intumescences produced by Atkinson on tomato (*1*) and by Douglas on potato (*6*) were clearly related in some way to excessive general sap pressure. They are not analogous cases to the root lenticels here considered, since the hypertrophy in the intumescences was, so far as can be judged from the illustrations given, mainly due to the stretching of soft tissue cells already present rather than to the formation of large masses of new cells.

It may be of some interest to note in passing that Cowles (*4, p. 553-554*) expresses himself as inclined to regard lacunar tissue in submerged parts of water plants to be a response to lack of transpiration rather than to oxygen deficiency.

The present writers' findings bearing on the factors causing hypertrophy of subterranean lenticels on young conifers are as follows:

1. Hypertrophy is apparently limited to trees with their roots in water or very wet soil. This may indicate either increased sap pressure or decreased aeration as among the effective stimuli. It seems rather improbable that there should be a significantly greater sap pressure in a mesophyte like *Pinus rigida* or a semixerophyte like *P. ponderosa* (Rocky Mountain type) in an excessively wet soil than in a plant in more normal condition. This seems especially improbable in view of the slow water absorption by the mesophytes in soil deficient in oxygen in the experiments already referred to (*12*).

2. While lenticel hypertrophy seems to be most common in soils containing clay, it has been frequently found in one nursery (at Halsey,

Nebr.) having a very sandy, well-drained soil, with a wilting coefficient[1] in the neighborhood of 3.4 per cent for the nursery as a whole, and an unusually high proportion of the soil (79 per cent) in particles between 0.25 and 0.05 mm. in diameter. The results of a mechanical analysis of this soil have already been published (*8*, *p. 2*). This, at first thought, indicates sap pressure rather than deficient aeration as the cause of hypertrophy. It is worthy of note, however, that in this case there was frequent artificial watering in addition to considerable rainfall, and it is therefore entirely possible that even in this case aeration was insufficient. Buckingham (*2*) found that both diffusion and molar movement of gas were slower in a wet sand than in any of the other soils, wet or dry, with which he experimented.

3. Reduction of the transpiring surface by removal of a large part of the needles, or of the terminal growth, or both, resulted in distinctly reducing the tendency to lenticel hypertrophy. (Table I.) The unpruned plants presumably had, at least part of the time, a lower general sap pressure than the pruned. The result of the experiment therefore tends to diminish the probability that there is any important causal relation between general excessive sap pressure and the hypertrophy in question.

4. The finding of the most abundant hypertrophy on roots which are deficient in fibrous laterals or whose absorbing surface has been greatly reduced by insect injury or by transplanting also tends to weaken the hypothesis that excessive general sap pressure throughout the plant is the chief cause of the hypertrophy. It is possible that roots which have little absorbing surface will take less oxygen from the soil than would better-developed root systems. An indication that this is the case is seen in the experience of Livingston and Free (*12*, *p. 185*) with the oxygen requirements of roots with different amounts of surface area. This association between deficient root surface and lenticel hypertrophy may therefore be an indication of a relation between oxygen deficiency and lenticel production.

The fact that lenticel hypertrophy was actually less in plants whose leaf surfaces had been reduced by pruning not only tends to decrease the probability of the "hyperhydrose" explanation; it is suggested that it is perhaps a further support for an oxygen-hunger (or carbon-dioxid excess) hypothesis. Plants with their leaf surfaces reduced during the latter part of the summer will of necessity produce less carbohydrate. The smaller amount of carbohydrate reaching the roots in consequence of the pruning might conceivably result in less respiration in the root tissues and therefore in a decreased need for oxygen. If this were the case the decreased oxygen hunger might furnish a partial explanation of the slight lenticel growth in the pruned plants.

[1] Determined by the Office of Biophysical Investigations, Bureau of Plant Industry.

Another possible connection between leaf pruning and oxygen hunger of root and stem is suggested by Prof. Livingston in a personal communication to one of the writers. A reduction of the transpiring surface by pruning should result in less absorption by the roots. If it be supposed that oxygen dissolved in water absorbed from the soil is important as a source of oxygen supply for the root tissues, a decrease in the amount of water absorbed might result in oxygen deficiency in the root tissues. This suggestion might help to explain the earlier reports of the stimulated growth of lenticels on stems of dicotyledons whose transpiration has been experimentally reduced. It obviously complicates any attempt to explain on an oxygen-hunger basis the effects of pruning on lenticel growth described in the present paper.

Of course it does not seem likely that any part of a plant accustomed to the presence of free oxygen would be likely to make much growth in the entire absence of oxygen. However, the condition existing in the soil in which the hypertrophies occurred certainly did not involve the entire absence of oxygen. Pfeffer concludes (*14, p. 115*), in spite of some conflicting evidence, that experiments have shown that reduction of the proportion of oxygen, at least in some cases, acts as an accelerating stimulus to growth.

It is of course true that any strong local growth is probably dependent on high local sap pressure. However, it is well known that such local high pressures are not necessarily dependent on excessive turgidity of the plant as a whole. Unusual chemical conditions, such as might conceivably result from local oxygen hunger, might easily cause them. The writers do not consider that oxygen hunger is established as the main cause of the lenticel hypertrophy found. They can not, however, agree with De Vaux in attributing the effect of increased soil moisture on lenticel growth entirely to increased water supply, excluding oxygen hunger as a possible factor in stimulating lenticel growth.

Experiments in which the oxygen, carbon-dioxid, and water supplies in the soil are independently controlled, as by the technic of Livingston and Free (*12*), and perhaps also with temperature control, will be needed to make a beginning on determining the relative importance of these various environmental factors in causing hypertrophy of root lenticels. Since conifers are rather difficult to handle in experimental work, poplar would perhaps be a better subject for preliminary experimentation. It seems likely, as has been suggested for hypertrophied lenticels in general by Tubeuf (*21*) and for intumescences by Hasselbring (*9*), that these unusual lenticel enlargements on the roots of conifers depend on a complex of conditions rather than on any one simple stimulus, and that with different species the conditions which call forth lenticel hypertrophy may be found to differ in relative importance.

RELATION BETWEEN LENTICEL HYPERTROPHY AND HEALTH OF PLANTS

Sorauer (*17, p. 210–219*) has used the name "tan disease" for lenticel hypertrophy on roots and stems of fruit trees. His use of the term "disease" appears justified in view of the association in many cases between the lenticel hypertrophy and a general pathological condition of the trees. The large lenticels described in the foregoing paragraphs as occurring on conifers are undoubtedly abnormal and in that sense are pathological. Since they occur only in abnormally wet situations, it is to be expected that in many cases the pines on which they have been found are unused to very moist surroundings and under the unfavorable conditions are subnormal in general vigor. The hypertrophies were first noted in a part of a nursery in which general vigor was unsatisfactory. Comparisons of the less vigorous and more vigorous plants in the section in which the hypertrophy was common showed lenticel hypertrophy present in both the weaker and stronger plants. The first examination, made by Hartley on about 200 3-year-old transplants of *Pinus ponderosa*, showed lenticel hypertrophy on a larger proportion of the weak trees than of the stronger trees. Later examinations made by Hahn on about 2,000 plants showed, particularly on *P. ponderosa*, that the greatest number of hypertrophied lenticels were associated with vigorous growth, This was true of plants in which the terminal root was rapidly advancing and the roots were large and stocky but correspondingly undeveloped as to lateral root surface. In one particular instance, however, where 2-year-old transplants of *P. ponderosa* had been badly affected by yellowing due to excessive irrigation, 50 per cent of 95 vigorous plants examined showed light occurrence of lenticel formation, while of 110 weakened and dying plants 80 per cent were found to exhibit light occurrence, and 10 per cent showed pronounced occurrence. This same bed examined a month later showed that the majority of the weak plants had died, while the vigorous plants, or those beginning to show renewed terminal growth, were alone showing freshly proliferating lenticels, those upon the dying plants becoming darkened and sloughing off. It therefore appears that lenticel hypertrophy is found on both weak and strong plants and that the conditions which bring on their formation may, if sufficiently prolonged, eventually cause the weakening and death of the plant. There is, however, so little direct connection between lenticel hypertrophy and the pathology of the conifers that it seems logical to recommend that any further investigation of the factors stimulating lenticel growth should be made from the point of view of physiology rather than from that of pathology.

SUMMARY

(1) Unusual excrescences on the roots of a number of different pines, spruces, and other conifers are found to have the structure of lenticels, much enlarged. They are produced in various kinds of soil in the presence of excessive moisture. Hypertrophy may occur on either weak or vigorous plants. Hypertrophy was decreased by top pruning and was increased by root injury. Such overgrowths have apparently not been previously reported on conifers.

(2) Conclusions of certain writers, based on work with dicotyledons, that excessive soil moisture stimulates lenticel hypertrophy mainly by increasing general sap pressure and that oxygen hunger is of no importance as a stimulus are not supported by the experience here set forth with conifers. Experiments in which the oxygen supply to the roots is varied without varying the water supply are believed necessary to settle the relative importance of these two factors.

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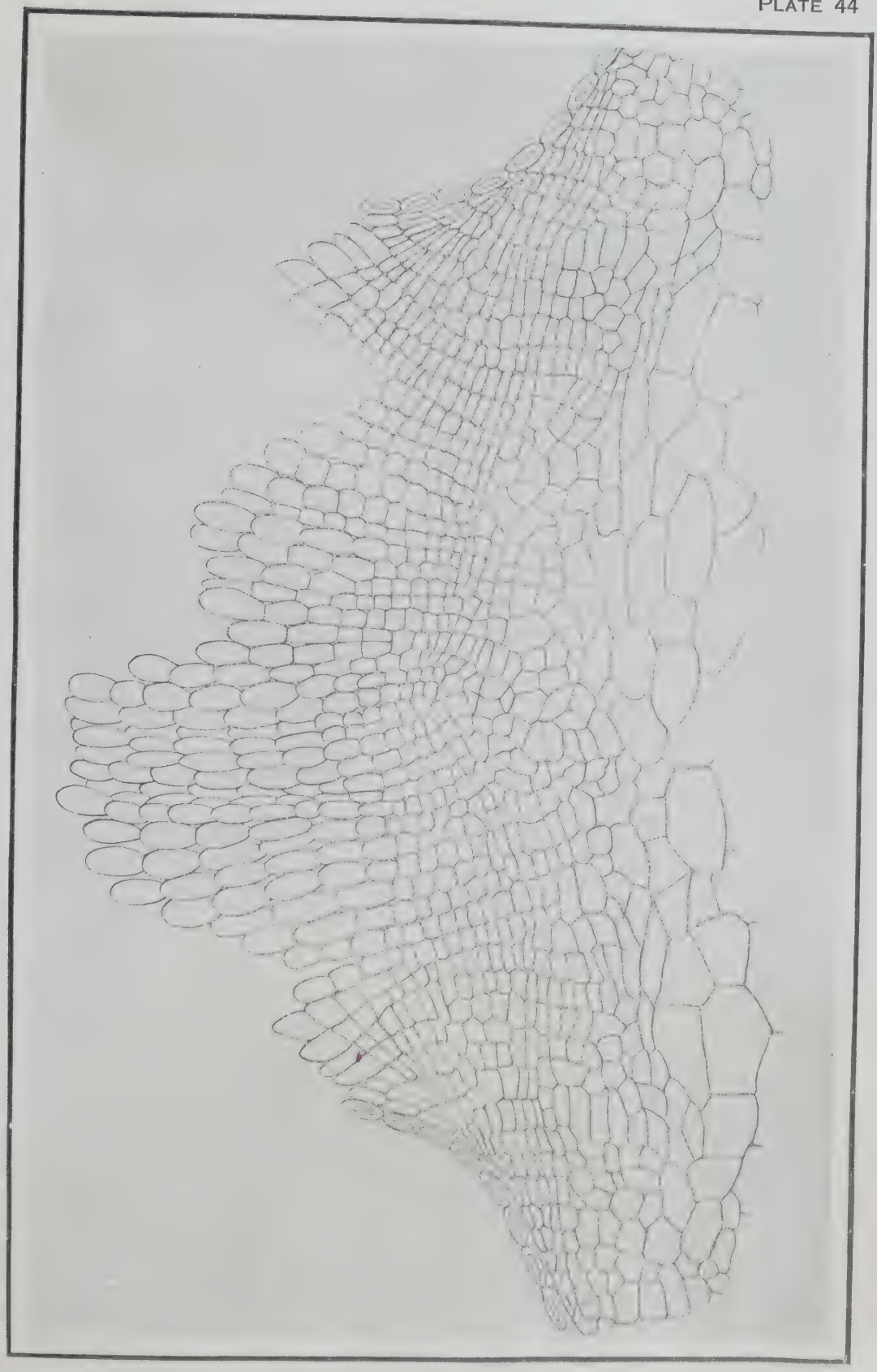
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PLATE 44

Section through a hypertrophied lenticel on root of *Pinus rigida* growing in swampy situation. Approximately × 59.




A
B

PLATE 45

A.—Hypertrophied lenticels on the basal part of layering stem of *Picea mariana*, which had been covered with sphagnum. Approximately × 1¾.

B.—Tap root of a *Pinus ponderosa* transplant, bearing an unusually large number of hypertrophied lenticels. Approximately × 1¾.

PLATE 46

A.—Cross section of the stem through one of the hypertrophied lenticels shown in C. In embedding and sectioning most of the loose outer tissues are unavoidably lost. Approximately × 112.

B.—Large patches of excrescences upon the tap root near the root crown, on *Pinus rigida*. Approximately × 1¾.

C.—Hypertrophied lenticels on root of 5-months-old *Pinus ponderosa*, grown in a loosely stoppered 2-ounce bottle, in tap water which had not been changed since the germination of the seed. The entire structure of the lenticel, which is too delicate to recover in digging roots from the soil, is here preserved. Approximately × 1¾.


A
B
C

DEGREE OF TEMPERATURE TO WHICH SOILS CAN BE COOLED WITHOUT FREEZING

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The general impression seems to be that when the temperature of soils falls slightly below the freezing point (0° C. or 32° F.) they freeze, that is, the soil moisture is converted into ice. This is hardly the case, however. In conducting investigations to study and measure the different forms of water in the soil by means of the dilatometer method[1] and to study and measure the concentration of the soil solution directly in the soil by means of the freezing-point method,[2] it was discovered that it is almost impossible to freeze the soils when they are cooled only slightly below the freezing point. This is true even when the concentration of the soil solution is exceedingly small and the freezing-point depression consequently negligible. Indeed, it was found that it is difficult to start solidification in the soils unless they are supercooled at about 1° C. below their true freezing point. Even at this degree of undercooling freezing begins only with vigorous agitation. If the soil is not vigorously agitated or disturbed it will remain at this temperature indefinitely without freezing. As the degree of undercooling is increased, however, the ease with which solidification is induced is also increased. Finally a temperature is reached where freezing starts automatically without agitation of the soil mass. This critical temperature is surprisingly low for all soils, as will be observed from the experimental data presented in Table I. This table shows the amount of cooling which the soils are able to withstand without freezing. The procedure by which these experimental results were obtained consisted in placing a 1-inch column of wet soil in a freezing-point tube, inserting the bulb of a Beckmann thermometer into this column of soil, and cooling the soil in different low temperatures until a temperature was reached where freezing would readily take place automatically. The figures represent approximately the limit of supercooling which these soils can resist without freezing. At this maximum degree of supercooling the soils can be maintained indefinitely if they are not disturbed or agitated. With a slight disturbance or agitation, however, they will

[1] BOUYOUCOS, George J. MEASUREMENT OF THE INACTIVE OR UNFREE MOISTURE IN THE SOIL BY MEANS OF THE DILATOMETER METHOD. *In* Jour. Agr. Research, v. 8, no. 6, p. 195–217, 1 fig. 1917.

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[2] ——— and MCCOOL, M. M. FURTHER STUDIES ON THE FREEZING POINT LOWERING OF SOILS. Mich. Agr. Exp. Sta. Tech. Bul. 31, 51 p. 1916.

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readily freeze. Again, if the temperature of cooling is only slightly lowered the soils will immediately freeze. These numerical data, therefore, represent just about the maximum cooling which the soils can withstand indefinitely without freezing when they are kept quiet.

For the sake of an interesting comparison, Table I also presents the limit of supercooling without freezing of several artificial materials.

TABLE I.—*The degree of cooling which soils and artificial materials can withstand without freezing when they are kept quiet and with the water content at about the saturation point*

Material.	Degree of supercooling without freezing.
	°C.
Quartz sand	−4.2
Coarse sand	−4.2
Fine sand	−4.2
Very fine sand	−4.2
Stony loam	−4.2
Loam	−4.2
Silt loam	−4.2
Clay loam	−4.2
Humus loam	−4.2
Clay	−4.2
Red clay	−4.2
Dark clay	−4.2
Brick clay	−4.2
Clay subsoil	−4.2
Peat	−5.0
Muck	−5.0
Water	−6.0
Silica	−6.0
Carbon black	−6.0
Gelatin	−6.0
Agar	−6.0

An examination of the foregoing experimental results reveals at once the fact that the amount of cooling which the soils are able to withstand without freezing is considerable, being about −4.2° C. (7.56°F.) for the mineral soils and about −5° C. (9° F.) for the peats and mucks.

It is of interest to observe that the maximum supercooling is still greater for the water and for the artificial materials, amounting in all cases to about −6° C. (10.8° F.). Since water freezes at about the same degree of supercooling as the artificial materials, it would logically seem that it is the water which limits the degree of supercooling of those materials and that they themselves have no influence on the degree of supercooling of water in one way or the other.

The question now rises, why do the soils withstand a smaller degree of supercooling than the artificial materials?

No definite explanation can be offered for this phenomenon. It would appear, however, that the true explanation is to be found in the difference in the size of particles of the two classes of materials. The artificial materials possess, of course, incomparably finer-sized particles than the soils do, and it would seem that when the division of a substance approaches the molecular state it ought not to affect the freezing of water materially. However, in a series of experiments conducted to ascertain if clay soils could withstand a greater degree of supercooling than coarse sands, it was found that sands with infinitely larger-sized particles resisted freezing equally as well as clays. It is possible, therefore, that other factors, such as the nature of the material, its cohesive and adhesive properties, its specific gravity, etc., also come into play in affecting the degree of supercooling.

In order to ascertain if the degree of moisture content exerts any influence upon the resistance of soils to freezing, different water contents were employed in all the various soils. The results failed to show, however, that moisture had any appreciable influence on the resistance of soils to freezing. Soils at a very low moisture content could not be supercooled any further than at a very high moisture content.

The foregoing experimental results afford a new and significant insight into the temperature of soils during the cold seasons. In the first place, they go to show that mineral soils may be cooled down to −4.2° C. (7.54° F.) and peats and mucks down to −5° C. (9° F.) without freezing. This being the case, the conclusion naturally follows that during mild winters and in mild climates in the winter the soils may not freeze even though they are cooled below their freezing point.

In the second place these findings prove quite conclusively that the method now in vogue for measuring temperature in soils in cold seasons may not give entirely the true facts. The thermometers will be recording the temperature to be several degrees below the freezing point and yet the soils may not be actually frozen.

The foregoing experimental results are very significant from still another standpoint. As it is well known, water in the liquid state has twice the specific heat that ice has. As long as the soil moisture remains in the liquid state the temperature fluctuations in the soil will be correspondingly slower and smaller.

Indeed, the ability of soils to resist freezing even when their temperature is much below the freezing point throws considerable new light on questions regarding the temperature of soils in cold seasons and consequently upon the physical, chemical, and bacteriological processes going on in the soils during those seasons.

CHANGES TAKING PLACE IN THE TEMPERING OF WHEAT

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In milling wheat it has been found advisable to "temper," "dampen," or "condition" the grain before grinding. This process consists in adding a certain amount of water to the wheat, then thoroughly mixing and allowing it to stand for a time. The treatment toughens the bran coat of the kernel, thus making possible a closer separation of the bran and the flour, and increases the desirable milling qualities of the wheat in other ways. The yield of flour is increased, and a flour is obtained from which better bread can be made. All practical millers are well acquainted with the fact that tempering improves the milling quality.

That Jago [1] recognizes the fact is shown by the following quotation:

> On making baking tests with the flours from such slightly dampened wheats, compared with those of the wheats milled dry, the dampened wheat flours fall off less during fermentation, yield bread of a better color and flavor, and in practically the same quantity. The slight damping of very dry wheats enables the miller to produce a better quality of flour.

Swanson[2] observes that conditioning not only toughens the bran of the wheat and makes it easier to crush the endosperm but it also affects the quality of the gluten and the baking quality of the flour. Temperature, moisture, and time play an important part in this process. Improvement through conditioning is similar to that brought about by natural ageing.

The changes in the flour are probably either physical or chemical, or more likely a combination of the two. The thorough elimination of the bran gives a flour of better color, and the closer separation of the bran and the endosperm produces a flour of higher gluten content. It is possible that the quality of the gluten is also affected. If so, this would indicate a chemical change during tempering or a physical change of such a nature as to make possible a more pronounced chemical change during fermentation and baking.

Since the experience of practical millers indicates that the physical changes mentioned above do occur, the subject is one which calls for accurate investigation. Millers often ask the question whether the obvious physical changes are accompanied by chemical changes. If so, a standardization of the factors which govern the tempering of wheat would lead to a more uniform product.

[1] Jago, William, and Jago, William C. TECHNOLOGY OF BREAD MAKING, p. 360. London, 1911.

[2] Swanson, C. O. WHEAT CONDITIONING. *In* Amer. Miller, v. 41, no. 6, p. 467-469, illus.

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The principal factors involved in the tempering of wheat are (1) time, (2) amount of water added, and (3) temperature. These factors vary somewhat with different varieties of wheat. The general practice of millers seems to be to temper from 12 to 48 hours and to add sufficient water to make the total moisture content 15½ per cent. There does not seem to be any fixed temperature used. Some millers pay no attention at all to this factor, while others "warm" the water before adding it to the wheat.

EXPERIMENTAL WORK

Three varieties or lots of wheat were used for the experimental work—a variety of hard wheat known as Kanred, developed recently by the Kansas Agricultural Experiment Station; a hard, red wheat (Turkey or Kharkof) from central Kansas; and a soft wheat from Colorado. This latter variety came to the department as Arizona White wheat.

The only chemical changes considered in this study were changes in the (1) hydrogen-ion concentration, (2) total acidity, (3) water-soluble phosphorus, and (4) titrable nitrogen. Yields of straight flour were also computed, and the milling qualities were judged as nearly as possible. Other investigations under way at the present time will be reported in a later paper.

Preliminary experiments were first conducted, from the results of which it seemed advisable to compare different periods of time, different temperatures, and different moisture contents as follows: (1) Time, 24 hours, 48 hours, and 72 hours; (2) temperature, 5°, 20°, and 40° C.; and (3) moisture content, 15½ and 18 per cent. The preliminary experiments seemed to indicate that the best results would be secured within these limits.

APPARATUS AND METHODS

The wheat was ground in a small burr mill driven by an electric motor. This mill was so made that it could be taken apart easily and cleaned. In addition, it was fitted with bran and flour sieves of silk bolting cloth.

The wheat was tempered and extracted in a large water thermostat fitted with a stirring device run by a small water motor. The thermostat was heated by a gas burner, and the temperature was kept constant (within 1° C.) by means of a mercury gas regulator.

The same hydrogen-ion apparatus was used as that described in a former paper,[1] excepting that the saturated potassium-chlorid electrode was used instead of the normal potassium-chlorid electrode.

The original moisture content of each lot of wheat was determined by drying in the air oven at 110° C. to constant weight. This was found to be 12.65 per cent for Kanred, 10.86 per cent for the Hard Red winter wheat, and 10.80 per cent for the Arizona White. In preparing the wheat and flour samples 200 gm. of wheat were weighed out into a 500-cc. bottle. To this was added sufficient distilled water to bring

[1] SWANSON, C. O., and TAGUE, E. L. DETERMINATION OF ACIDITY AND TITRABLE NITROGEN IN WHEAT WITH THE HYDROGEN ELECTRODE. *In* Jour. Agr. Research, v. 16, no. 1, p. 1–13, 6 fig. 1919.

the total moisture content up to the desired percentage. The bottle was corked tightly and the mixture was well shaken. The bottle was then placed in the thermostat, which had been brought to the desired temperature, and the mixture was allowed to remain in the thermostat for the desired length of time. At the end of the time the wheat was ground as rapidly as possible in the mill. The mill was set to grind to the same fineness for each lot of wheat, and each lot was put through the mill the same number of times. During the grinding the milling qualities were judged as nearly as possible, and after grinding the yields of straight flour were calculated.

Sufficient flour to equal 50 gm. on a moisture-free basis was immediately weighed out. This was placed in a fruit jar, and sufficient distilled carbon-dioxid-free water was added to make the ratio of moisture-free flour to water 1 to 10. This water had been previously heated to 40° C. To this mixture 2 cc. of toluene were added as a preservative, the jar was tightly closed by means of a rubber and a screw cap, and the contents were thoroughly mixed by shaking. The jar was then placed in the thermostat, where the temperature was 40° C. The flour was extracted for 2 hours at this temperature, the jar being well shaken every 15 minutes. At the end of 2 hours the jar was removed and the contents were poured into a centrifuge cup. The cup was then placed in the centrifuge and whirled for 5 minutes at a speed of 2,500 revolutions per minute. Finally the supernatant liquid was poured through a folded filter, and the filtrate was used for the determinations of hydrogen-ion concentration, total acidity, water-soluble phosphorus, and titrable nitrogen. For the determination of the hydrogen-ion concentration and total acidity 100 cc. of the filtrate were pipetted into an electrode vessel. The vessel was then placed in the hydrogen-ion apparatus, and hydrogen gas was passed through until the potential remained constant (within 1 millivolt) for 15 minutes. During the entire time the vessel was shaken 60 times per minute. After this constant potential was noted, *N/10* alkali was run in from a burette in small portions at a time until the constant potential indicated a P_H value of 7, which is the absolute neutral point. The number of cubic centimeters of *N/10* alkali used were then taken to represent the total acidity.[1]

The water-soluble phosphorus was determined from a second 100-cc. portion from the same filtrate. The phosphorus was determined by the usual method after the organic matter had been destroyed by boiling with nitric acid. The titrable nitrogen was determined in a third 100-cc. portion by the formaldehyde method of Sorensen, using thymolphthalein as an indicator. The number of cubic centimeters given in Table I multiplied by 1.4 gives the number of milligrams of titrable nitrogen in 100 cc. of the extract.

For a control, a portion of each variety of wheat, untempered, was ground, and an extract was made of each in exactly the way described

[1] For fuller description see SWANSON, C. O., and TAGUE, E. L. OP. CIT.

above. The same determinations were then made on these extracts as on the tempered lots.

The results for each variety of wheat are presented in Tables I, II, and III.

TABLE I.—*Yield of flour, hydrogen-ion, concentration, total acidity, water-soluble phosphorus, and titrable nitrogen of the flour from Kanred wheat*

Time tempered.	Temperature.	Hydrogen-ion concentration.	Total acidity.[a]	Water-soluble phosphorus.	Titrable nitrogen.[a]	Yield of flour.[b]	Remarks.
Hours.	°C.	P_H.		*Per cent.*			
.....	.....	6.20	1.7	0.0361	4.2	68	Brittle and hard.
24.....	5	6.20	1.7	.0359	4.2	67	Ground fairly well.
48.....	5	6.22	1.8	.0360	4.1	69	Do.
72.....	5	6.20	1.7	.0361	4.3	68	Do.
24.....	20	6.17	1.7	.0361	4.4	70	Do.
48.....	20	6.19	1.7	.0362	4.6	72	Ground well.
72.....	20	6.13	1.8	.0380	4.5	71	Do.
24.....	40	6.06	2.0	.0376	4.6	72	Do.
48.....	40	6.00	2.0	.0379	4.6	72	Do.
72.....	40	6.00	2.0	.0378	4.8	70	Sticky.

[a] Expressed as number of cubic centimeters $N/10$ sodium hydroxid required to titrate 10 gm. flour.
[b] Expressed as number of grams of flour obtained from 100 gm. of wheat.

TABLE II.—*Yield of flour, hydrogen-ion concentration, total acidity, water-soluble phosphorus, and titrable nitrogen of the flour from Hard Red winter wheat (Turkey or Khorkof)*

Time tempered.	Temperature.	Hydrogen-ion concentration.	Total acidity.[a]	Water-soluble phosphorus.	Titrable nitrogen.[a]	Yield of flour.[b]	Remarks.
Hours.	°C.	P_H.		*Per cent.*			
.....	.....	6.13	1.9	0.0469	3.6	65	Brittle and hard.
24.....	5	6.13	1.8	.0460	3.8	67	Somewhat softer.
48.....	5	6.15	1.9	.0468	3.7	67	Do.
72.....	5	6.10	1.9	.0472	3.8	68	Do.
24.....	20	6.06	1.9	.0472	3.9	68	Ground fairly well.
48.....	20	6.06	1.9	.0471	3.8	69	Do.
72.....	20	6.06	1.9	.0476	4.0	70	Ground well.
24.....	40	5.92	2.2	.0479	4.0	71	Do.
48.....	40	5.91	2.1	.0483	4.1	70	Do.
72.....	40	5.92	2.2	.0482	4.0	68	Sticky.

[a] Expressed as number of cubic centimeters of $N/10$ sodium hydroxid required to titrate 10 gm. flour.
[b] Expressed as number of grams of flour obtained from 100 gm. of wheat.

TABLE III.—*Yield of flour, hydrogen-ion concentration, total acidity, water-soluble phosphorus, and titrable nitrogen of the flour from Arizona White wheat*

Time tempered.	Temperature.	Hydrogen-ion concentration.	Total acidity.[a]	Water-soluble phosphorus.	Titrable nitrogen.[a]	Yield of flour.[b]	Remarks.
Hours.	°C.	P_H.		*Per cent.*			
.....	.....	5.89	1.5	0.0177	2.0	67	Brittle.
24.....	5	5.92	1.5	.0176	1.9	67	Ground well.
48.....	5	5.92	1.5	.0176	1.9	68	Do.
72.....	5	5.89	1.5	.0179	2.1	68	Do.
24.....	20	5.89	1.6	.0179	2.1	70	Do.
48.....	20	5.86	1.6	.0181	2.2	72	Do.
72.....	20	5.86	1.7	.0182	2.1	70	Do.
24.....	40	5.82	1.7	.0186	2.3	71	Do.
48.....	40	5.82	1.8	.0185	2.3	70	Sticky.
72.....	40	5.79	1.8	.0184	2.1	69	Do.

[a] Expressed as number of cubic centimeters of $N/10$ sodium hydroxid required to titrate 10 gm. flour.
[b] Expressed as number of grams of flour obtained from 100 gm. of wheat.

The addition of sufficient water to make the total moisture content 18 per cent was tried with each variety of wheat. In every case the resulting flour was sticky, the sieves became clogged, and the yields were reduced below that for the untempered grain. For this reason the analyses of the flour from this treatment were not completed.

It will be noted that when the wheat was tempered at 5° C. there was practically no chemical change as compared with the untempered wheat. As a general rule the yields were slightly higher and the milling qualities were considerably better than those secured from the control or untempered wheat. In each case the bran was tougher, and a cleaner separation of the bran and endosperm was possible. The length of time appeared to have very little influence on either the physical or chemical composition of the flour.

When the wheat was tempered at 20° C., a small but definite chemical change took place. The hydrogen-ion concentration was increased, as was shown by a lower P_H value. The total acidity, the water-soluble phosphorus, and the titrable nitrogen were also higher. Both the yield and the milling quality were better than when the wheat was tempered at 5° C. The time of tempering appeared to be a factor in the chemical changes but had very little if any relation to the physical qualities.

The chemical changes were still more pronounced when the grain was tempered at 40° C. The physical changes appeared to be detrimental to the milling qualities of the grain. In other words, increasing the time of tempering increased the chemical changes but proved detrimental after 48 hours so far as the milling value of the wheat was concerned.

In general the milling qualities of the drier wheats were improved by tempering more than were those of the wetter wheats, and the hard wheats were improved more than the soft wheats.

It may be concluded from these experiments that slight chemical changes take place during the tempering process and that these changes increase with time and temperature. Improvement in the milling qualities is confirmed also, excepting in cases where the time of tempering exceeded 48 hours and where the temperature exceeded 20° C. It would appear from this that (1) the improved milling quality of tempered wheat is due chiefly to physical changes, (2) a temperature of 20 to 25° C. is best, (3) 15½ per cent moisture appears to be about the best, (4) the maximum improvement takes place in 48 hours, (5) hard wheats are improved more than soft wheats, and (6) dry wheats are improved more than wet wheats.

VASCULAR DISCOLORATION OF IRISH POTATO TUBERS

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INTRODUCTION

The exact significance of vascular discoloration in the stem-end tissues of Irish potato tubers has never been fully determined. Various types of both flesh and vascular necrosis are recognized, some of which are associated with the presence of Fusaria of various species or with *Verticillium albo-atrum*. Others, however, at least in the initial stages, yield no organisms when subjected to culture, nor does the microscope reveal the presence of organisms. It is also recognized that a superficial necrosis may develop in the stem tissues of apparently perfectly normal stock. There is no such perfect natural abscission of the potato tuber from the stolon as is common with fruits. Moreover, they are frequently harvested before the plants are mature, and the tubers are then broken off from green stolons. It has been assumed that suberization of the wound thus made normally follows in two or three days, so that not more than a few layers of dead cells should appear unless some aggressive parasite gains entrance to the wound. A popular impression has prevailed that any except the most superficial stem-end discoloration might be taken as a trustworthy indication of the presence of Fusarium, or, at least, that the stock was grown on vines affected with Fusarium or Verticillium.

Somewhat extensive preliminary observations and cultural studies, made by the writer both at the time of harvest and during or at the close of the rest period, on stock grown in sections where Fusarium blight and wilt do not occur, as well as in sections where they are known to be general, show that, while Fusarium and Verticillium undoubtedly do cause vascular discoloration of potato tubers, discoloration can not be accepted as proof of the presence of Fusarium or, indeed, of any other organism, nor can the absence of discoloration be confidently accepted as proof of the sterility of the vessels near the stolon attachment. There seems to be reason to think that vascular necrosis may often arise from purely physiological causes and that it need not necessarily be seriously abnormal, though frequently it is. A more complete discussion of this question must await the outcome of studies at present incomplete, but it seems advisable to present some available data regarding the fungous flora of potato stem ends.

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The notes from which these data have been compiled were obtained jointly by Venus W. Pool, M. B. McKay, H. G. MacMillan, R. D. Rands, and the writer during the spring and summer of 1915. The writer wishes to make full acknowledgment to these associates and to assume the entire responsibility for the construction placed on the notes and the deductions made from them, as well as for the accuracy of the tabulations and compilations presented.

OUTLINE OF METHODS AND WORK

The general plan followed in the work may be outlined briefly as follows: Material for experimental plantings, involving about 4 acres of plots, was secured from various sources, as reported below. It was treated 30 minutes in 1 to 1,000 mercuric chlorid solution and allowed to dry, after which each tuber was examined for vascular discoloration by removing with a flamed and cooled scalpel a shallow cone of tissue with the stolon attachment at the center of its base. A record was made of the presence or absence of discoloration and of the general character of the discoloration when present, as slight, medium, brown, dark, etc. When discoloration was found, the depth to which it penetrated in the tuber was determined by removing a wedge of tissue. When browning was confined to a shallow area around the removed cone it was designated by recording the symptom A. If the discoloration extended to a greater depth, involving up to one-fourth the length of the tuber, symptom B was recorded. A deeper discoloration was designated by C. Discolored tubers were submitted to culture. In general one planting of tissue was made from each region involved in discoloration. As a rule, therefore, one planting was made from tubers showing symptom A, two from those showing symptom B, three from tubers showing symptom C, and none from those showing no discoloration. In the actual prosecution of the work, however, certain deviations from the general rule were introduced, either to check the dependability of results or to secure additional information. The tubers of each lot were weighed and numbered consecutively in the order of their respective weights, which were recorded. With the exception of lot No. 3, the tubers of each lot weighing less than 3 ounces were divided into two groups, one comprising all the even numbers and the other all the odd numbers. Those weighing 3 ounces or more were halved from stem to apex, one half being placed with the small tubers of even number and the other half with the small tubers of odd number. When the half tubers weighed 3 ounces or more they were cut into stem and apex portions. In a few cases the half tubers were so large as to yield stem, middle, and apex pieces, or even stem, two middle, and apex pieces—four in all from each half. The minimum seed piece for cut tubers was 1½ ounces.

The two lots of seed stock were planted and grown in widely separated regions and under distinctly different environmental conditions of soil and climate, one lot being planted on a light, sandy soil, under rainfall, at Waupaca, Wis., and the other on a heavy clay loam under irrigation at Greeley, Colo. The identity of each plant was preserved, and frequent records were made by the same observers in rotation in each and in both regions to secure all the data possible regarding the influence of the seed piece and environment and of the interrelations of these upon individual plant performance, with special reference to the development of pathological conditions.

DESCRIPTION OF MATERIAL

The material may be divided advantageously for consideration into three groups, each containing several lots. The first group comprises stock affected with tuber-borne diseases of undertermined origin; the second lot is from healthy parentage; and the third is from diseased parentage where the malady is regarded as of parasitic origin. For brevity in presentation many lots which were held separate during the investigation have been combined, so as to appear as a unit, whenever their origin and performance made such treatment feasible.

A brief description and index of the lots presented in the tables follows.

A.—OBSCURE DISEASE GROUP.

1. Thirty-four seedling varieties originated by Prof. Wm. Stuart, of the Department of Agriculture, and originally regarded as promising but ultimately discarded because of the persistent reappearance of destructive but imperfectly understood hereditary diseases. This material had been grown at Jerome, Idaho, in 1913 and 1914, in the pathological plots there.

2. The progeny of 31 hills of Western Peach Blow, grown at Greeley, Colo., which were suspected of Fusarium infection. This stock is now known to be affected also with leafroll and mosaic and is therefore placed in this group.

3. A miscellaneous collection of 21 lots from the pathological collection of the field station at Presque Isle, Me. Both seedling and commercial varieties affected with leafroll, mosaic, and dwarfing diseases were included. This lot was grown only at Greeley, Colo., and the tubers were either planted whole, or, if they weighed over 3 ounces, they were cut once crosswise into stem and apex halves.

B.—HEALTHY GROUP.

4. A representative commercial lot of the variety Late Ohio, grown at Greeley, Colo., in 1914 and obtained from the grower.

5. An exceptionally good commercial strain of the variety Pearl, grown in Greeley, Colo., in 1914, obtained from the grower and collected from the field at harvest time.

6. A fine commercial strain of the variety Pearl, grown at Crandon, Wis., in 1914 and reported to be free from wilt, leafroll, and similar diseases.

7. Wisconsin certified seed potatoes, variety Pearl, secured from the grower.

8. Culls from two lots of Maine-grown stock of the variety Pearl. One of these lots was reported healthy and the other as diseased with leafroll. There was no difference in the performance of the two lots in either locality where they were grown, and disease was absent. They are therefore grouped together as healthy.

9. Certified seed potatoes of the variety Rural New Yorker, grown at Boss Lake, Wis. A second lot of similar, though uncertified, material of the same variety but from another grower near Racine, Wis.

10. A small lot of Wisconsin-grown stock of the variety Pearl, composed of tubers on the stolons of which Colletotrichum pycnidia were developing.

11. Four so-called types of commercial stock of the variety Rural New Yorker, supplied by a local grower of Greeley, Colo., who had used his own home-grown seed for a series of years. These types were really only rather imperfectly established size grades, evidently obtained by bin selection from the general field run of his stock.

C.—Parasitic disease group.

12. The progeny of representative hills from a typical "Fusarium-blight" field of the variety Early Ohio, grown at Greeley, Colo., in 1914, dug in August and stored in a mass lot.

13. Ten hill lots of the variety Early Ohio, grown at Greeley, Colo., in 1914. The physical condition of the soil of the field was poor, and the plants were small and dwarfed.

14. A representative lot from a field of choice stock of the variety Sir Walter Raleigh, grown in 1914 on a field at East Lansing, Mich., which was heavily infected by Fusarium. Every plant in the field, with the exception of about one-quarter of 1 per cent, wilted and died three or four weeks before frost.

15. Sixty-one hill lots of the variety Pearl, grown in Wisconsin in 1914. The hills selected were from vines with more or less rolled foliage and a brown discoloration of the vascular tissue of the stems. Cultures from the discolored stem tissue failed to yield Fusarium.

16. Eighteen hill lots of the variety Pearl, grown from Wisconsin seed at Greeley, Colo., in 1914. Cultural tests at digging time showed unusual infection of the vines with *Fusarium oxysporum*.

17. Six hill lots of the variety Red McClure, grown at Greeley, Colo., in 1914 on vines shown by isolations to be infected with *Fusarium oxysporum*.

18. Forty hill lots of the variety Rural New Yorker, grown on diseased vines at Waupaca, Wis., in 1914. Cultural tests of the vines for Fusarium at digging time yielded a Fusarium and a Colletrotrichum culture in about equal numbers, but these did not appear to be general.

19. Twenty-five hill lots of the variety Rural New Yorker, grown at Greeley, Colo., in 1914 on vines infected with *Fusarium oxysporum*, as shown by isolation tests from the vascular tissue of the stems at digging time.

PRESENTATION OF RESULTS

VASCULAR DISCOLORATION

The number of tubers in each lot of material and the number having discolored vascular bundles, grouped according to the relative depth of penetration below the stolon attachment, are shown in Table I. A column for miscellaneous symptoms is included to provide for a variety of incidental occurrences, such as net necrosis, decay, mechanical injury, and the like; and following this, the number of tubers of each lot with no vascular discoloration is shown.

It has already been stated that, in general, tubers with stem-end vascular tissue of normal appearance were not submitted to culture and that one, two, or three cultures were made from tubers with discolored vessels, the actual number being determined by the depth of the necrosis. No regular procedure was adopted with respect to the tubers belonging to the miscellaneous group. The figures in the column marked "theoretical," under "number of cultures," have been obtained by adding the number of shallow discolorations, twice the number of deep discolorations, three times the number of very deep discolorations, and whatever number the notes show to be correct to provide the cultures made from tubers with miscellaneous symptoms. The actual number of cultures made and reported upon follows in the next column. Under "duplicates" are included the number of cultures made from discolored tubers in excess of the number theoretically required. The number of cultures made from tubers with no discoloration of the stem-end tissue is next recorded, and last of all is given the number of cases in which a culture was theoretically called for but was not reported. In some cases, for one reason or another, these cultures were not made, while in others they were made and discarded before being studied. because of broken tubes, loss of identifying label, and similar accidents. If the number given in the last column is subtracted from the sum of the numbers in the two preceding columns and the difference is added to the theoretical number of cultures, the actual number is obtained.

TABLE I.—*Appearance of vascular tissue and origin of cultures*

Lot No. and designation.	Number of tubers.	Nature of discoloration.					Number of cultures.				
		Shallow.	Deep.	Very deep.	Miscellaneous.	None.	Theoretical.	Actual.	Duplicate.	From tubers not discolored.	Theoretically required but lacking.
OBSCURE DISEASE GROUP											
1 Id.....	1,731	474	26	4	5	1,222	544	590	44	57	55
2 WPB.....	387	159	1	1	2	224	164	162	8	13	23
3 Me.....	636	206	2	0	10	418	217	215	6	14	22
HEALTHY GROUP											
4 CLO.....	335	233	21	2	9	70	290	373	85	5	7
5 PC.....	957	563	0	0	2	392	564	572	19	11	22
6 PW.....	537	80	0	1	5	451	84	89	1	12	8
7 PSW.....	65	10	0	0	0	55	10	9	0	0	1
8 PMe.....	133	14	0	0	0	119	14	16	0	2	0
9 RW.....	360	58	0	0	1	301	58	59	2	5	6
10 RWColl..	7	0	0	0	7	0	7	7	0	0	0
11 CRC.....	664	262	3	0	2	397	269	280	13	9	11
PARASITIC DISEASE GROUP											
12 AEO....	212	132	17	0	0	63	166	181	24	2	11
13 EO.....	69	17	1	1	0	50	22	22	1	1	2
14 M.....	546	289	6	1	1	249	305	298	10	8	25
15 DPW....	391	85	0	0	3	303	88	80	1	7	16
16 DPC.....	152	88	0	0	0	64	88	106	16	5	3
17 RMc.....	47	14	0	0	0	33	14	16	1	2	1
18 DRW...	222	51	0	0	0	171	51	55	0	5	1
19 DRC.....	145	61	3	0	0	81	67	73	8	3	5
Total.....	7,596	2,796	80	10	47	4,663	3,022	3,203	239	161	219

ISOLATION AND IDENTIFICATION OF FUNGI

Isolations were made by transferring a small piece of tissue removed under aseptic conditions from the region of discoloration directly to a test tube containing sterilized nutrient material prepared in the usual way. Melilotus stems, potato cylinders, and steamed rice were used, the number of each diminishing in the order named. Identifications were made direct from the original tube in some cases, while subcultures were resorted to in others. Except in part of the Fusarium cultures, no attempt was made to identify the species. Two hundred and ninety-one out of the 718 cultures of Fusarium secured were identified as *F. discolor* var. *sulphureum* or *F. oxysporum*, but it is not to be supposed that the remaining 499 cultures were all of other species. Indeed, it is probable that *F. oxysporum* and *F. radicicola* predominated among the cultures reported as *Fusarium* spp. The summarized results of the cultural studies are presented in Table II. Two columns of figures appear under each genus reported. In the first column is given the number of instances when the culture was either pure or so nearly so as not to give

visible evidence of the presence of other organisms at the time of identification. In the second column is recorded the number of times the genus in question was found in a tube associated with some other organism. Each tube containing a mixed culture is reported twice, once for each organism. In no case were more than two organisms identified from a single tube. The total number of identifications reported is therefore the sum of all the columns marked "pure" plus the sum of all the columns marked "mixed," while the total number of plantings reported is the sum of all the columns marked pure plus one-half the sum of all the columns marked "mixed."

One very significant thing shown in Table II is the fact that out of 3,203 plantings, all but 161 of which were made from discolored tissue, 1,352 gave no growth. There is good reason to believe that in the great majority of these cases the tubes yielded no growth because the tissue transplanted was sterile, or at least free from filamentous fungi. These results are in entire accord with those obtained by the writer in numerous other cases where cultural tests of discolored vascular tissue of potatoes have been carried out. In some instances the discoloration may be a response to parasitic attack on some other portion of the plant, though the tissues of the tuber are not actually attacked. In such cases it may be regarded as a parasitic phenomenon of a secondary character. From the physiological point of view, however, it matters little whether a lethal dose of toxin diffuses from some point in the stem back of the stolon or from a point within the tuber itself. Likewise, the result is the same whether the tissue is killed by the action of fungi, primary or secondary, or through the operation, directly or indirectly, of malign environment of whatever nature. Conclusions based on field experiments with many factors uncontrolled must not be accepted without reserve, but the writer has secured deep vascular discoloration which he believes to be the direct result of too rapid respiration induced in the soil at high temperatures such as prevail during the summer months in the vicinity of Washington and which are occasionally experienced at more northern and western points. This was the case with stock grown at Arlington Farm during the summer of 1917, in which vascular discoloration was universal and pronounced, extending throughout the tuber in most cases. While certain lots of this material yielded Fusarium or other fungi from a certain portion of the plantings, other lots yielded only an occasional saprophytic growth out of hundreds of plantings. The results were confirmed by repeated trials, which gave uniformly identical results.

There seems, therefore, to be good reason to regard some of the stem-end browning of vascular tissue as physiological, even in the cases in which it extends well into the tubers.

TABLE II.—*Number of isolations from stem-end tissue*

Lot No.	*Alternaria* spp.		*Bacterium* spp.		*Colletotrichum* spp.		*Fusarium discolor sulphurium.*		*Fusarium oxysporum.*		*Fusarium* spp.		*Penicillium* spp.		*Rhizoctonia* spp.		*Verticillium* spp.		Miscellaneous.		No growth.	Total.	
	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.		Pure.	Mixed.
OBSCURE DISEASE GROUP																							
1	59	3	26	6	0	1	11	0	8	0	25	6	20	0	3	1	6	5	18	0	403	579	22
2	62	3	17	0	1	0	4	1	1	0	14	2	13	0	0	0	9	1	4	1	33	158	8
3	30	6	2	0	4	6	1	0	0	0	3	1	6	1	0	0	0	0	7	0	155	208	14
HEALTHY GROUP																							
4	25	9	7	4	1	0	4	0	29	0	185	13	2	1	0	0	2	1	5	0	99	359	28
5	183	19	31	4	8	4	12	0	6	0	11	5	47	1	1	0	67	18	12	1	168	546	52
6	12	1	36	2	0	2	1	0	6	1	10	4	0	0	1	0	1	0	1	0	16	84	10
7	1	0	0	6	0	0	0	0	2	2	0	4	0	0	0	0	0	0	0	0	0	3	12
8	3	1	1	0	2	0	0	0	0	0	2	0	0	0	1	0	0	0	0	1	6	15	2
9	7	0	6	7	2	2	1	0	14	1	6	8	0	0	1	0	0	0	3	0	10	50	18
10	0	0	0	2	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	4	5	4
11	11	3	24	3	1	0	8	1	8	1	57	2	4	0	2	0	7	1	9	1	143	274	12
PARASITIC DISEASE GROUP																							
12	90	15	3	1	0	0	3	0	8	0	40	14	1	0	0	0	1	0	1	2	18	165	32
13	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	18	22	0
14	0	0	2	3	8	44	1	0	59	43	0	3	0	0	1	0	8	1	18	0	154	251	94
15	13	0	19	5	2	0	2	0	23	0	0	5	0	0	0	0	2	0	0	0	14	75	10
16	43	3	6	1	2	0	1	0	0	0	2	0	4	0	1	0	1	2	2	0	41	103	6
17	2	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	5	1	1	0	5	15	2
18	5	0	12	1	0	0	0	0	26	0	0	1	0	0	0	0	0	0	0	0	11	54	2
19	3	1	4	0	0	0	1	0	0	0	2	1	1	1	0	0	6	1	0	0	54	71	4
Total	550	65	196	45	32	59	51	2	190	48	358	71	100	4	11	1	116	31	81	6	1,352	3,037	332
Total for genus	.....	615	.....	241	.....	91	.....	53	.....	238	.....	429	.....	104	.....	12	.....	147	.....	87	1,352	.....	3,369

Another striking thing brought out in Table II is the frequency with which Alternaria was recovered from the vascular tissue. Almost 20 per cent of the discolored tubers carried this genus, in most instances unmixed with other fungi. This proportion is so high as to suggest that it may possess some significance hitherto unsuspected or at least undiscovered. Similar results have frequently been secured with other material. As high as 50 per cent of some lots of tubers have yielded Alternaria in cultural tests, even from stock presenting an attractive appearance on superficial examination.

FIELD STUDIES

The general manner in which the stock was handled in planting has already been indicated (p. 277–278). In taking notes in the field a full description of each plant was recorded at each reading, including such matters as size, habit, character, color, and orientation of stems and foliage, as well as the general appearance as to vigor. At least three sets of notes, and in the case of some lots more, were made on each plant during the season. Successive sets of notes were taken by different members of the staff, and no reference to the previous notes was made while preparing the new set. In the preparation of the present article the writer has endeavored to translate these descriptions into the expressions "diseased" and "healthy." Every plant has been placed in one group or the other, even though in some cases the assignment had to be more or less arbitrary. Consistency has been maintained, however, and the writer has been able to bring to his aid thorough familiarity with the appearance of the material throughout the season. One of the three principal sets of notes is his own.

Plants whose description at any given note taking indicates probable suspicion in the mind of the observer of the presence of disease have been recorded as diseased, even though at previous or subsequent note takings they may be recorded as healthy. It is certain that many cases of recorded disease at the first note taking represent only delayed germination, but as this may be correlated with reduced vitality or fungous attack on the sprout or tuber, it seems important to record it. Records of recovery as well as of disease have been made and will be considered later, but it is of interest first to inquire into the general relation of vascular discoloration to fungous invasion and the correlation of these within the tuber with disease in the plants produced. For the purpose of this consideration plants once reported as diseased have been counted as diseased whether later reported as diseased or healthy.

RELATION OF VASCULAR DISCOLORATION TO FUNGOUS INVASION AND DISEASE

Table III is designed to show the performance in the field of all the tubers studied, arranged according to the character of the tubers. The tubers are grouped under four headings:

1. Tubers with vascular discoloration yielding a culture.
2. Tubers with vascular discoloration yielding no culture.
3. Tubers without vascular discoloration yielding no culture.
4. Tubers without vascular discoloration yielding a culture.

The tubers under each heading are arranged in two columns, according as they yielded plants which were healthy or diseased. In case a tuber was cut into two or more pieces at least one of which produced a diseased plant, the tuber has been reported in the disease column. As is to be expected, most of the plants in the progeny of the lots carrying obscure tuber-borne diseases are diseased. The results presented in the remaining two groups, however, seem to indicate that vascular discoloration does not necessarily imply fongous invasion; nor is either of these in the tuber a guarantee of disease in the plant, or their absence a guarantee of health.

TABLE III.—*Number of healthy and diseased plants from tubers examined*

OBSCURE DISEASE GROUP

Lot No.	Discoloration; fungus present.		Discoloration; fungus absent.		No discoloration; fungus absent.		No discoloration; fungus present.		Total.
	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	
1	15	147	23	324	86	1,124	0	12	1,731
2	22	90	14	37	42	171	2	9	387
3	8	46	22	142	73	339	2	4	636
Total	45	283	59	503	201	1,634	4	25	.....

HEALTHY GROUP

4	128	65	53	19	55	10	4	1	335
5	263	118	131	53	261	122	6	3	957
6	28	33	12	13	249	190	8	4	537
7	5	4	1	0	22	33	0	0	65
8	8	0	6	0	114	3	2	0	133
9	19	25	6	9	151	146	2	2	360
10	3	0	0	4	0	0	0	0	7
11	82	46	74	65	216	175	5	1	664
Total	536	291	283	163	1,068	679	27	11	.....

TABLE III.—*Number of healthy and diseased plants from tubers examined*—Continued

PARASITIC DISEASE GROUP

Lot No.	Discoloration; fungus present.		Discoloration; fugus absent.		No discoloration; fungus absent.		No discoloration; fungus present.		Total.
	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	
12	113	18	16	2	52	9	2	0	212
13	2	5	4	8	34	15	1	0	69
14	76	62	76	83	133	113	2	1	546
15	20	39	7	22	115	182	0	6	391
16	17	43	10	18	29	31	2	2	152
17	3	6	3	2	12	20	1	0	47
18	17	25	4	5	93	74	1	3	222
19	5	12	23	24	29	50	0	2	145
Total	253	210	143	164	497	494	9	14	.....
Grand total	834	784	485	830	1,766	2,807	40	50	7,596

INFLUENCE OF ENVIRONMENT

Influence of environment upon the development of disease and recovery is a subject of much interest and importance. Table IV brings out some interesting facts regarding the development of disease in Wisconsin and in Colorado in cut and uncut seed. It should be borne in mind that the plants grown in the two States from cut seed are from the same individual, since, as has already been stated, the tubers were halved lengthwise and one half was planted in each place. The seed under 3 ounces was not cut but was divided into two approximately equal portions for planting. For the cut seed the total number of tubers cut and the total number of seed pieces appear in each line. One-half the number of seed pieces is the number planted in each State, except from lot 3. This lot was planted in Colorado only, and it was halved crosswise into stem and apex pieces instead of lengthwise.

The third and fourth columns give the number of diseased plants developing in Wisconsin and Colorado, respectively, and the following column gives the number of cases in which corresponding portions of a given tuber yielded diseased plants in both places. These plants are referred to as pairs. In No. 3 only, the pairs are from stem and apex halves of the same tuber. Of the 197 diseased plants recorded, 106 were from stem-end seed pieces and 91 were from apex or seed ends. As shown in the table, 79 pairs occurred.

For the uncut seed the number of tubers planted in each State and the number developing disease in each State appear.

TABLE IV.—*Distribution of diseased plants*

OBSCURE DISEASE GROUP

Lot No.	Cut seed.					Whole seed.			
	Number of tubers.	Number of seed pieces.	Number of diseased plants in Wisconsin.	Number of diseased plants in Colorado.	Number of diseased pairs.	Number of tubers in Wisconsin.	Number of diseased plants in Wisconsin.	Number of tubers in Colorado.	Number of diseased plants in Colorado.
1	757	1,636	729	698	650	483	432	491	454
2	11	22	8	9	7	194	181	182	116
3	143	286	.....	197	79	.....	.....	493	414
Total	911	1,944	737	904	736	677	613	1,166	984

HEALTHY GROUP

4	184	376	52	45	25	75	12	76	11
5	631	1,552	105	201	42	163	24	163	42
6	482	1,076	177	141	62	27	4	28	3
7	46	114	17	35	12	9	3	10	3
8	10	20	0	0	0	60	1	62	2
9	300	718	104	153	64	30	11	30	5
10	7	14	3	2	1	0	0	0	0
11	84	202	10	73	8	290	82	290	142
Total	1,744	4,072	468	650	214	654	137	658	208

PARASITIC DISEASE GROUP

12	23	46	1	2	0	96	13	93	13
13	5	10	0	1	0	34	13	30	14
14	484	1,136	113	197	52	30	11	32	8
15	125	272	56	74	41	121	75	145	91
16	28	56	19	17	14	63	41	62	31
17	15	30	10	4	3	17	13	15	4
18	96	214	40	37	17	52	25	74	25
19	61	150	31	45	23	45	14	39	26
Total	837	1,914	270	377	150	458	205	490	212
Grand total	3,492	7,930	1,475	1,931	1,100	1,789	955	2,315	1,404

The figures given in Table IV indicate no conspicuous relation between the character of the tuber used for seed and the occurrence of disease, since the number of pairs of diseased plants is only equal to from one-half to one-third the total number of diseased plants in either locality. It is to be noted that in general the Colorado conditions resulted in more disease than did those of Wisconsin, particularly when cut seed was used, and this, too, notwithstanding the fact that the cut tubers were well suberized when planted.

These results seem to indicate that the soil and not the tubers should be considered the most potent source of disease, a fact substantiated for the Greeley section by the more recent studies of Dr. MacMillan. Additional indication of this probability is given in Table V, where the behavior of stem and apex seed pieces is presented and the number of diseased plants per tuber is shown. The obscure disease group, of course, shows a majority of cases in which all the plants from a tuber were diseased, when any of them were; but the combined results from the healthy and the parasitic disease groups show that out of 283 quartered tubers yielding diseased plants, 123 yielded 1 such plant only, 99 yielded 2, 33 yielded 3, while only 28 yielded 4.

TABLE V.—*Number of tubers yielding diseased plants*

Lot No.	From stem pieces.	From apex pieces.	From both stem and apex pieces.	Total number of tubers.	Total number of tubers yielding diseased plants.	Number of tubers yielding 1 diseased plant.	Number of tubers yielding 2 diseased plants.	Number of tubers yielding 3 diseased plants.	Number of tubers yielding 4 diseased. plants.
OBSCURE DISEASE GROUP									
1	51	51	49	[a] 57	53	3	5	3	42
2	.....	.....	.....	0	.....	.....	.....	.....	.....
3	106	91	79	[b] 143	118	39	79	.....	.....
HEALTHY GROUP									
4	1	1	0	[c] 4	2	1	1	0	0
5	71	33	27	[d] 141	77	44	20	5	8
6	48	25	23	[c] 56	50	17	18	12	3
7	11	9	9	[c] 11	11	1	6	1	3
8	.....	.....	.....	0	.....	.....	.....	.....	.....
9	46	23	21	59	48	20	14	11	3
10	.....	.....	.....	0	.....	.....	.....	.....	.....
11	16	13	12	[c] 17	17	5	12	0	0
PARASITIC DISEASE GROUP									
12	.....	.....	.....	0	.....	.....	.....	.....	.....
13	.....	.....	.....	0	.....	.....	.....	.....	.....
14	41	26	18	[c] 84	49	24	16	3	6
15	9	7	6	11	10	2	6	0	2
16	.....	.....	.....	0	.....	.....	.....	.....	.....
17	.....	.....	.....	0	.....	.....	.....	.....	.....
18	6	3	3	11	6	2	3	0	1
19	9	10	6	[c] 14	13	7	3	1	2

[a] Two tubers were cut into eight pieces each. All yielded diseased plants. Other tubers were cut into four pieces each.

[b] Tubers were cut into two pieces each.

[c] Tubers were cut into four pieces each.

[d] Four tubers were cut into six pieces each. All produced healthy plants, except one stem and one middle piece from the same side of one tuber. These are both recorded as stem plants. Other tubers were cut into four pieces.

It appears further from the data given on the second and third groups in Table V that a tuber from healthy parentage or from fungous-invaded parentage is more likely to yield a diseased plant from a stem-end seed piece than from the apex. Two hundred and fifty-eight tubers yielded diseased plants from stem ends and 150 yielded diseased plants from apex ends. One hundred and twenty-five of these tubers yielded diseased plants from both stem and apex. The ratios, therefore, of stem, apex, and pairs were approximately 10 : 6 : 5. The fact that the proportion of diseased stem plants to diseased apex plants is slightly higher in the healthy group than in the parasitic disease group is not inconsistent with other data presented in this paper.

The facts seem to indicate that the greater liability of stem-end plants to disease results not because the vascular tissue of the seed piece is more often infected by fungi but because it is more often endowed with less physiological resistance.

DISEASE AND RECOVERY

Data dealing with disease and recovery are presented in Table VI. The total number of plants reported at the first note taking as diseased is recorded in the first column. Following this is recorded the number of these plants which subsequently appeared to recover and to remain healthy. The next column gives the number of additional plants reported diseased at the second note taking, followed similarly by the number of those which subsequently recovered. The next column records the number of hitherto healthy plants which appeared to be diseased at the third note taking.

In the lower portion of the table the Rural New Yorker and the Pearl varieties have been summarized in juxtaposition for purposes of convenient comparison. The outstanding feature of this table is the remarkable degree of recovery shown, particularly in Colorado. This is especially noticeable with the Pearl stock in Colorado. It is, possibly, the ability of the Pearl to recuperate in that section which accounts for the popularity of this variety in the Greeley region.

A summary of the data on disease and on recovery for the entire experiment in total and by States is given in Table VII. Table VIII shows percentage data figured from information shown in Tables IV and VII. Attention is directed to the figures in Tables IV and VII in connection with the percentage averages in Table VIII, because percentage figures may be misleading when the numbers from which they are computed are small. A striking example of this is shown in Table VIII, where one plant in Wisconsin was diseased and did not recover, while two were diseased in Colorado and both recovered. This appears in the respective columns on recovery as 0 and 100 per cent. In the larger groups and in the aggregates, however, reduction to percentage gives a clearer presentation of the facts.

TABLE VI.—*Disease and recovery*

OBSCURE DISEASE GROUP

Lot No.	Number of diseased plants.	Wisconsin.					Colorado.					Number not recovered.	
		Number in first note taking.		Number added in second note taking.		Number added in third note taking.	Number in first note taking.		Number added in second note taking.		Number added in third note taking.		
		Diseased.	Recovered.	Diseased.	Recovered.	Diseased.	Diseased.	Recovered.	Diseased.	Recovered.	Diseased.	Wisconsin.	Colorado.
1	2,313	943	67	170	31	48	824	97	263	24	65	1,063	1,031
2	314	15	1	112	15	62	93	73	32	32	0	173	20
3	611	.....	.....	.....	.....	.....	372	41	237	29	2	.....	541
Total	3,238	958	68	282	46	110	1,289	211	532	85	67	1,236	1,592

HEALTHY GROUP

4	120	34	11	19	3	11	53	23	0	0	3	50	33
5	372	29	14	54	16	46	177	113	28	14	38	99	116
6	325	67	18	13	5	101	127	50	13	8	4	158	86
7	58	2	1	18	13	0	32	12	5	3	1	6	23
8	3	0	0	1	0	0	2	2	0	0	0	1	0
9	273	55	26	51	41	9	135	74	22	18	1	48	66
10	5	2	1	0	0	1	2	2	0	0	0	2	0
11	307	3	1	75	62	14	208	182	4	3	3	29	30
Total	1,463	192	72	231	140	182	736	458	72	46	50	393	354

PARASITIC DISEASE GROUP

12	29	14	12	0	0	0	5	3	9	6	1	2	6
13	28	1	1	9	0	3	3	3	1	1	11	12	11
14	329	26	8	87	63	11	184	143	12	4	9	53	58
15	296	25	2	65	3	41	35	22	125	101	5	126	42
16	108	35	1	6	0	19	34	13	8	3	6	59	32
17	31	1	0	22	7	0	2	2	6	3	0	16	3
18	127	20	4	44	35	1	43	24	16	16	3	26	22
19	116	6	0	38	12	1	55	49	2	1	14	33	21
Total	1,064	128	28	271	120	76	361	259	179	135	49	327	195
Healthy Pearl	758	98	33	86	34	147	338	177	46	25	43	264	225
Diseased Pearl	404	60	3	71	3	60	69	35	133	104	11	185	74
Total	1,162	158	36	157	37	207	407	212	179	129	54	449	299
Healthy Rural New Yorker	585	60	28	126	103	24	345	258	26	21	4	79	96
Diseased Rural New Yorker	243	26	4	82	47	2	98	73	18	17	17	59	43
Total	828	86	32	208	150	26	443	331	44	38	21	138	139

TABLE VII.—*Summary of disease and recovery*

OBSCURE DISEASE GROUP

Lot No.	Colorado and Wisconsin. Number of tubers.	Number of seed pieces.	Number of diseased plants.	Number of recovered plants.	Wisconsin. Number of seed pieces.	Number of diseased plants.	Number of recovered plants.	Colorado. Number of seed pieces.	Number of diseased plants.	Number of recovered plants.
1	1,731	2,610	2,313	219	1,301	1,161	98	1,309	1,152	121
2	387	398	314	121	205	189	16	193	125	105
3	636	779	611	70	.....	.....	.....	779	611	70
Total	2,754	3,787	3,238	410	1,506	1,350	114	2,281	1,888	296

HEALTHY GROUP

4	335	516	120	37	263	64	14	264	56	23
5	957	1,878	372	157	939	129	30	939	243	127
6	537	1,131	325	81	565	181	23	566	144	58
7	65	133	58	34	66	20	14	67	38	15
8	133	275	3	1	70	1	0	72	2	2
9	360	778	273	159	389	115	67	389	158	92
10	7	14	5	3	7	3	1	7	2	2
11	664	782	307	248	391	92	63	391	215	185
Total	3,058	5,507	1,463	720	2,690	605	212	2,695	858	504

PARASITIC DISEASE GROUP

12	274	235	29	21	119	14	12	116	15	9
13	69	74	28	5	39	13	1	35	15	4
14	546	1,198	329	218	598	124	71	600	205	147
15	391	538	296	128	257	131	5	281	165	123
16	152	181	108	17	91	60	1	90	48	16
17	47	62	31	12	32	23	7	30	8	5
18	222	340	127	79	159	65	39	181	62	40
19	145	234	116	62	120	45	12	114	71	50
Total	1,784	2,862	1,064	542	1,415	475	148	1,447	589	394
Grand total	7,596	12,156	5,765	1,672	5,611	2,430	474	6,423	3,335	1,194
Healthy Pearl	1,692	3,417	758	273	1,640	331	67	1,644	427	202
Diseased Pearl	543	719	404	145	348	191	6	371	213	139
Total	2,235	4,134	1,162	418	1,988	522	73	2,015	640	341
Healthy Rural New Yorker	1,031	1,574	585	410	787	210	131	787	375	279
Diseased Rural New Yorker	367	574	243	141	279	110	51	295	133	90
Total	1,398	2,148	828	551	1,066	320	182	1,082	508	369

TABLE VIII.—*Summary of disease and recovery in percentage*

OBSCURE DISEASE GROUP

Lot No.	Percentage diseased.						Percentage recovered.	
	Cut seed.		Whole seed.		All seed.			
	Wisconsin.	Colorado.	Wisconsin.	Colorado.	Wisconsin.	Colorado.	Wisconsin.	Colorado.
1	89.12	85.33	89.44	92.46	89.24	88.01	8.44	10.52
2	72.73	81.82	93.30	63.74	92.20	64.77	8.47	84.00
3	.....	66.88	.....	83.98	.....	78.43	.....	11.46
Total	88.90	81.08	90.55	84.39	89.64	82.77	8.44	15.69

HEALTHY GROUP

4	27.66	23.94	16.00	14.47	24.33	21.21	21.88	41.07
5	13.53	25.90	14.72	25.77	13.74	25.88	23.26	52.26
6	32.90	26.21	14.81	10.71	32.04	25.44	12.71	40.28
7	29.82	61.40	33.33	30.00	30.30	56.72	70.00	39.47
8	0.00	0.00	1.67	3.23	1.43	2.78	0.00	100.00
9	28.97	42.62	36.67	16.67	29.56	40.62	58.26	58.23
10	42.86	28.57	0.00	0.00	42.86	28.57	33.33	100.00
11	9.90	72.28	28.28	48.97	23.53	54.99	68.48	86.05
Total	22.99	31.93	20.95	31.56	22.49	31.84	35.04	58.74

PARASITIC DISEASE GROUP

12	4.35	8.70	13.54	13.98	11.76	12.93	85.71	60.00
13	0.00	20.00	38.24	46.67	33.33	42.86	7.69	26.67
14	19.89	34.68	36.67	25.00	20.74	34.17	57.26	71.71
15	41.18	54.41	61.98	62.76	50.97	58.72	3.82	74.55
16	67.86	60.71	65.08	50.00	65.93	53.33	1.67	33.33
17	66.67	26.67	76.47	26.67	71.88	26.67	30.43	62.50
18	37.38	34.58	48.08	33.78	40.88	34.25	60.00	64.52
19	41.33	60.00	31.11	66.67	37.50	62.28	26.67	70.42
Total	28.21	39.39	44.76	43.27	33.57	40.70	31.16	66.89
Grand total	38.59	47.01	53.38	60.65	43.31	51.92	19.51	35.82
Healthy Pearl	21.65	27.30	12.36	19.01	20.18	25.97	20.24	47.31
Diseased Pearl	45.73	55.48	63.04	58.94	54.89	57.41	3.14	65.26
Total	24.21	30.29	33.41	36.60	26.26	31.76	13.98	53.28
Healthy Rural New Yorker	25.05	48.82	29.06	45.94	26.68	47.65	62.38	74.40
Diseased Rural New Yorker	39.01	45.05	40.21	45.13	39.43	45.08	46.36	67.67
Total	28.97	47.77	31.65	45.73	30.02	46.95	56.87	72.64

SUMMARY

In the material studied, vascular discoloration of stem-end tissues of Irish potato tubers was not found to be proof of the presence of parasitic fungi. Discolored bundles were often sterile, and fungi were frequently isolated from tissues which appeared normal.

The organisms recovered, in the order of their greatest frequency, were Fusarium 720, Alternaria 615, bacteria 241, Verticillium 147, Penicillium 104, Colletotrichum 91, Rhizoctonia 12, miscellaneous 87.

Out of 3,203 plantings, all but 161 of which were from discolored tissues, 1,352 gave no growth.

The field trials indicate that neither vascular discoloration nor fungus invasion of the tissues of the mother tuber is a guarantee of disease in the resulting plants, nor is their absence a guarantee of health. The soil and not the tuber appeared to have been the more potent source of disease.

Stem-end seed pieces yielded slightly higher percentages of disease than eye-end pieces, evidently because the stem end is endowed with less physiological resistance.

The plants showed a marked capacity for recuperation, which varied with the variety, with the environment, and with the interaction of the two.

CROWNWART OF ALFALFA CAUSED BY UROPHLYCTIS ALFALFAE

By FRED REUEL JONES, *Pathologist*, and CHARLES DRECHSLER, *Assistant Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

When between the years 1909 and 1914 the so-called crownwart of alfalfa was found scattered through several important alfalfa-growing regions on the Pacific slope of the United States, much interest was aroused. The earliest publication dealing with the disease in South America indicated that it might become of considerable economic importance. The fact that it had attained but limited distribution suggested that prompt study might reveal the possibility of effective measures against further spread as well as means of averting serious loss in the regions already invaded. In 1915 this interest formulated itself in a petition [1] framed by the American Phytopathological Society addressed to the United States Department of Agriculture calling attention to existing conditions and urging work upon this interstate problem. In 1917 it became one of the duties of the senior author to begin work upon this disease. The junior author was associated with the work in 1919, making the field observations, giving especial consideration to the taxonomy and morphology of the causal organism, and preparing all the drawings. This paper is a report of the progress that has been made in the study of this disease.

THE DISEASE

COMMON NAMES

In the United States the disease is commonly know by either of two names, crowngall and crownwart. As will be shown later, the structure of the diseased tissue is that of a true gall, and it was called such in the earlier reports of its occurrence. Later the name crownwart was suggested in order to distinguish the disease from the bacterial crowngall caused by *Pseudomonas tumefaciens*, though it had not then been shown that this disease occurs upon alfalfa in the field. Recently, however, galls have been found by Mr. H. L. Westover on alfalfa in Arizona which appear to be true crowngalls, though complete proof is lacking. In view of the fact that a gall similar in appearance to that caused by *Urophlyctis alfalfae* (Lagerh.) P. Magnus is found upon alfalfa, it is even

[1] Phytopathology, v. 5, no. 2, p. 130–131. 1915.

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more desirable than formerly that the disease caused by Urophlyctis should have a distinctive name. The fact that the name crownwart is well established in usage is much in its favor. It will be seen, however, from facts presented later in this paper that this name is somewhat misleading, inasmuch as the galls are not typical warty growths, nor are they formed from the tissue of the so-called crown of the plant in a manner comparable with that in which crowngalls are formed. A name more truly distinctive is suggested by the French name used by Arnaud (*1*),[1] "La Maladie des tumeurs marbrèes de la Luzerne." An English equivalent, marbled gall of alfalfa, the word marbled referring to the mottled effect produced by the brown spore masses seen when any of these galls are cut, would call attention to the one distinctive character of these galls observable at any time and would be accurately descriptive.

HOST PLANTS

Of the many species of the genus Medicago introduced into the United States, *Medicago sativa* is the only one on which the disease has been found commonly. McKee (*17*) found it also on *M. falcata*. The two species, grown near together at the Plant Introduction Field Station at Chico, Calif., seemed to be about equally infected.

Spegazzini (*32*) records the fungus as occurring on *Medicago denticulata* and species of Adesmia in Argentina. Hauman-Merck (*11*) also records the fungus on *M. denticulata* from the same locality and further states that it does not occur upon alfalfa. In view of the fact that search has not revealed the fungus upon *M. denticulata* in the United States even when the plant is growing abundantly close in association with diseased alfalfa, it seems advisable to hold it an open question whether the fungus found in Argentina upon *M. denticulata* and *Adesmia* spp. is identical with that which causes the disease of alfalfa.[2] Thus, the evidence at hand, while it is inadequate for the formation of final conclusions, appears to indicate that the species of Urophlyctis occurring on *M. sativa* is probably limited to that species and to *M. falcata*.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The only available information regarding the economic importance of the disease consists of expressions of opinion based on a larger or smaller amount of field observation. The trend of the opinion that has developed from this observation is that the disease is, or becomes locally, very destructive to alfalfa plants.

The first report of the disease by von Lagerheim (*14*) from Ecuador gave inception to this trend. He states that diseased plants can easily

[1] Reference is made by number (italic) to "Literature cited," pp. 321–323.

[2] A portion of a collection of *Urophlyctis alfalfae* var. *adesmiae* on *Adesmia bicolor*, sent by Spegazzini to the Office of Pathological Collections, Bureau of Plant Industry, has been examined and been found to contain a Synchitrium rather than a Urophlyctis.

be distinguished in the field, and his illustrations of diseased plants with crowns encrusted with large galls contributed effective support to his statements. However, von Lagerheim states that he did not see the disease in the field himself, though he sought for it in fields near Quito. He received his specimens from the owner of an estate in the Andes, and his description of the effects of the disease in the field was gathered from several observers.

In Europe, Magnus (*20*) reports a destructive outbreak of the disease in Alsace, basing his report on the observations of two farmers. Later, from an adjoining Province of Germany, Grimm and Korff (*10*) report the disease as present in an alfalfa field without causing much apparent harm. In fact, the diseased plants seemed somewhat more vigorous than the others. Nevertheless, they think measures should be taken to eliminate it.

Peglion (*25*) finds the disease in Italy, and raises the question whether or not it may be a factor in producing alfalfa sickness in some fields. He suggests that experimental work should be undertaken to determine the matter. In France Arnaud (*1*) reports the disease as apparently doing considerable damage in a single field in the Department of Seine-et-Oise. In 1916 Salmon (*27*) found a single field infested with wart in England and urged further search for the disease. No reports of serious infestations have followed, though the writers have been told that occasional specimens are found. The disease has been found in Holland (*8*) and Sweden, but no apparent damage has been reported.

A critical reading of these reports of the destructive action of the disease calls attention to the fact that the two most important reports, those of von Lagerheim and of Magnus, are not based on first-hand observation. In all cases damage is noted only in small areas. Therefore we must still hold it an open question whether this disease has been primarily responsible for any serious or widespread injury to alfalfa in either South America or Europe.

In the United States the disease has been found abundant only west of the Sierra Nevada and Cascade Mountains, though it occurs in a few regions east of these mountains. It has not been found east of the Rockies. However, in view of the fact that the disease when not abundant is often completely concealed unless a plant is uprooted, it is possible that its distribution is more widespread than records show.

The first report of the disease by Smith (*31*) gives no clue to its importance. O'Gara (*22*) finds the disease very common and occasionally destructive in fields in the Rogue River Valley in Oregon. Jackson (*12*) later reports the disease from the same region, making no comment regarding its importance. Again O'Gara (*23*) is first to report the disease present in the Salt Lake Valley in Utah, though he has not in this case determined to what extent it causes injury. McCallum (*16*) reported the disease present in Arizona. McKee (*17*), who has had an opportunity

to observe the disease extensively, concludes that crownwart decreases the yield and shortens the life period of plants. He says:

> Alfalfa fields that had the crown wart in abundance in 1914 produced good crops of hay in that year and in 1915. In one field sown in 1910 that has been under observation the past two years, practically every plant has galls. This field has produced apparently normal crops of hay, but more critical observation shows decreased vigor in the plants and a corresponding decrease of yield.

McKee believes the disease much more widespread than is commonly supposed and urges work to determine its importance in alfalfa culture.

Thus it appears that although the disease is scattered through large alfalfa-growing areas in the United States, yet it does not appear at any place to have become regarded as a serious limiting factor in the growth of the crop, except during years of severe attack and even then in small areas.

The writers have not attempted to determine the present limits of spread of the disease in the United States. A limited amount of time has been spent in the spring of three years observing the disease, chiefly in the river valleys where it is known to be most abundant, the Sacramento River Valley in California and the Rogue River Valley in Oregon. The second of these years, 1918, appears to have been distinctly unfavorable for the development of the disease, especially in California. The winter rainfall was below normal, in consequence of which the Sacramento River did not overflow its flood plain where McKee observed the disease to be most abundant. The disease was commonly present on a larger or smaller percentage of plants, but nowhere did observation bring conviction that considerable damage was being done. In the San Joaquin Valley that year only occasional diseased plants could be found, though in some localities there was excellent testimony from farmers of the abundance of the disease in previous years.

In 1919 there was much more winter rain, especially in the Sacramento River Valley, and a greater amount of disease was found. Even then it was only rarely that the disease was sufficiently abundant to appear to be of serious economic importance. Plants could be found whose early buds had become so completely infected that few were left to form the second and later cuttings, but such plants were usually widely scattered among others less severely infected. Rarely indeed does the disease appear to be solely responsible for the killing of entire plants, though it must often weaken them. A significant estimate of the actual damage done can be made only after careful observation has extended over a period of years when the varying intensity of the annual attacks can be studied and the behavior of the diseased plants followed throughout the year.

DESCRIPTION OF THE DISEASE (PL. 47)

The disease is more easily described by stating briefly the origin and method of development of the galls. So far as the writers can discover

all galls as they occur naturally in the field result from the infection of buds in early stages of development as they emerge from the crown of the plant. It is well known that there is an almost continuous succession in the development of buds from the so-called alfalfa crown during the entire year. A portion of those buds which will produce the shoots furnishing the first crop in the spring have begun development as early as the preceding autumn. Generally speaking, the first buds to be formed in the seasonal succession have a point of origin deeper in the soil than those which are formed later, so that many of the buds from which the shoots of the third cutting arise develop from positions quite above the surface of the soil. Buds produced below the soil level in cool weather appear to have a meager protection of scaly covering, and it is for the most part such buds that become infected and give rise to galls. Thus, galls are swollen and distorted bud elements, scales, leaves, and stipules.

Unless overwintered galls which are described later are discoverable, the disease is first evidenced in the spring by a slight thickening and rounding of the young buds. During two years this has been observed near Chico, Calif., in the latter part of March or early in April. The diseased buds become more and more rounded as growth progresses and are glistening white in color (Pl. 54, A). Then, as the infected structures begin to push apart, some of them grow much more rapidly than others until the structure as a whole assumes a conspicuously irregular form. In most cases, however, an examination of the gall will show that it is made up of thick, scalelike layers about a central growing axis (Pl. 36, B). Sometimes this axis continues growth in spite of the demands of the mass of developing gall tissue and produces a weak shoot. The earlier and more vigorous buds produce the larger galls. Smaller galls often appear to be developed from smaller buds along the stems below ground that would ordinarily remain dormant. The origin of galls that appear on stems several inches above the surface of the ground in wet weather appears to be due in part to the infection of axillary buds that would never develop in the ordinary course of events and in part to the elongation of the stems and petioles which force infected tissue upward.

Since a large part of the infected buds are developed at a depth of 2 or 3 inches below the surface of the soil, the majority of the galls are so far below ground that they escape observation unless the soil is removed from around the plant. If they are of large size some of them come to the surface, where they take on a green color and in extreme cases form a crust of diseased tissue around the base of the healthy stems.

Another type of gall that is not common results from local infections on young leaves. Such infections give rise to small blister-like galls much like those produced on *Sanicula* spp. by another species of this fungus which will be mentioned later.

The galls reach full development (Pl. 54, B) early in the summer, in early June in northern California. From this time on the majority of them begin to decay if moisture is abundant, or to shrivel and dry with the coming of drouth. However, in almost all fields a few galls more deeply situated become covered with a corky layer and survive the winter.

When plants are subjected to dry conditions in late summer, as they usually are in the Rogue River Valley in Oregon, many of the galls do not decay but remain living throughout the autumn and winter. It does not appear that such galls make appreciable growth during the following year. Nevertheless, gall tissue may accumulate around old plants in considerable mass. The exterior becomes covered with a brown, corky layer that has a much warted appearance. This accumulation of gall tissue has not been found on plants that have grown in well-irrigated fields.

At whatever age or state of development these galls are found, they possess one distinctive character that is discovered when they are cut open. The interior of the galls contains many small, irregularly shaped brown masses of fungus spores which are easily visible (Pl. 56, B). In old dried galls the host tissue has shrunken so much that the spore mass often occupies a large portion of the mass of the gall. Even in decayed galls that have not yet been broken to fragments the spore masses can be recognized by their golden brown color.

CAUSAL ORGANISM

NOMENCLATURE

Some difference of opinion concerning the identity of the parasite causing crownwart of alfalfa has prevailed. Von Lagerheim (*24*) seems first to have regarded it as a new and distinct species, which he cited as *Cladochytrium alfalfae*. Later, however, he (*14*) identified it with *Urophlyctis* (*Physoderma*) *leproidea*, a parasite causing conspicuous malformations on the beet, originally described from Algeria by Trabut (*34*) and assigned by him as well as by Saccardo and Mattirolo (*26*) to a new Ustilaginous genus, Oedomyces. In making this disposition, von Lagerheim opposed the views of both Vuillemin (*35*), who had identified Trabut's beet organism with *Urophlyctis* (*Cladochytrium*) *pulposa* (Wallroth), long known to be parasitic on species of Chenopodium and Atriplex, and of Magnus (*18–20*), who later came to regard the parasites on *Chenopodium* spp., on the beet, and on alfalfa as three distinct species. None of these views appear to be based on evidence altogether conclusive; nor can we adduce such evidence here, because the lack of fresh diseased material of beet and of *Chenopodium* spp. have made it impossible to attempt cross-inoculation experiments.

Provisionally, it appears advisable to follow Magnus in recognizing the alfalfa parasite as a distinct species, not, perhaps, so much on account of some differences in morbid host anatomy as because of the general improbability that two unrelated plants serve as hosts to a parasite which shows in general no omnivorous tendencies. The beet disease has not been reported in the regions where crownwart is prevalent; and *Chenopodium* spp. with every chance for infection have not been observed to be attacked. Reference has been made in another connection to Spegazzini's (*32*) report of crownwart on *Medicago denticulata* and its absence from alfalfa in the same range. This condition could most readily be attributed to the existence of another species producing similar galls.

DEVELOPMENT AND MORPHOLOGY OF THE FUNGUS

The morphology of the crownwart organism has not hitherto received much attention. Magnus (*20*) made some observations regarding enlarged hyphae frequently found in old material and referred to the presence of a hyaline cell attached to the concave side of the resting spores; but in the main his specific details concern the pathological anatomy of the host. In more recent years, Wilson (*37*) published a cytological account of *Urophlyctis alfalfae*, arriving at conclusions considerably at variance with those of Magnus. The utilization of old material by both these writers may largely account for their failure to observe important details of development and morphology, as well as explain interpretations that it appears impossible to reconcile with conditions as found in young material much more favorable for study.

GERMINATION OF THE RESTING SPORES

As has long been recognized, the fungus passes through the prolonged periods of summer drouth by means of the resting spores contained within cavities in the galls of the host. In the course of the rainy season the galls disintegrate completely, thus setting free the spores; and it is not improbable that the exposure incident to this method of liberation may be necessary for germination. However, the conditions that may favor germination remain more or less obscure; for although many attempts were made by the writers with spores from freshly gathered material both old and young, as well as with limited supplies of material that may, in addition, have suffered deterioration in transit, the results obtained have been so meager and dubious that this phase of the life history of the fungus must be reserved for a later paper. In a number of preparations an appearance was noted as of resting spores producing a number of subspherical bodies varying from 1 to 9, by the passage of protoplasm through pores in the spore wall. The vesicles that usually attained half the linear dimensions of the spore in some cases were seen

to produce endogenous motile bodies resembling zoospores that later escaped through a number of openings on the distil side of the vesicular wall. As the Van Tiegham cultures in which this process was noticed were usually several days old, the development of bacteria and various protozoa brought into the observations a considerable measure of untrustworthiness. Indications that similar contaminations may have affected the observations of Wilson (*37*) on *Urophlyctis alfalfae* and of Bally (*2*) on *U. rübsaameni* are not entirely wanting. Both of these writers describe the resting spore as functioning directly as zoosporangium.[1]

PENETRATION OF THE HOST

Because of difficulties encountered in efforts to bring about infection under artificial conditions, it has not been possible to observe directly the penetration of the host by the germinating zoospore. However, as an abundance of conditions immediately following the entrance of the parasite were found in stained sections of buds, the course of events during the time of invasion can be followed in incipient stages in the same manner as during advanced stages.

Bodies measuring 3 to 4 μ in diameter were frequently found attached or adhering to the scales or developing axis of the bud. They appear to have made their way under the bud scales very close to the most rapidly growing meristem. Unfortunately, no clear figures showing the immediate development of these bodies were observed—a failure attributable apparently to the fact that by the time the galls became noticeable many weeks had seemingly elapsed since the period during which infection took place abundantly. As a result, the earliest demonstrable stage of invasion was represented by the presence of small turbinate bodies (the "Sammelzellen," "corps centrals," "vésicules collectrices," or "vésicules collectives" of other writers) within the epidermal cells of the outer foliar or scale elements of buds exposed to attack, and attached to and perforating the cuticular wall by an elongated beak (Pl. 49, A, *ta–tg*). More than one body may be present in the same epidermal cell, two or three being not unusual; and occasionally a considerable number of contiguous cells may show such evidence of multiple and concentrated attack. The beak manifestly represents the tube proliferated by the zoospore through which the contents of the latter were conveyed into the host cell after the manner prevailing very generally throughout the Chytridiales.

[1] In an article that has appeared since this paper was prepared, Wilson (*38*) gives a more detailed account of his findings. So far as his account concerns the germination of the resting spores, it appears to differ very considerably from that more recently published by C. Emlen Scott (*30*), according to whom each resting spore proliferates from 1 to 15 sporangia, the zoospores escaping through a number of tubes in the hyaline wall. With the latter account the observations recorded above are not at variance

GROWTH OF THE PARASITE

The fungus cell thus produced is first uninucleated and bears at its apex a short, cylindrical projection. As it becomes older it increases in size, the single nucleus divides, giving rise to a multinucleated condition, and the short apical projection proliferates more or less successively three or four terminal branches which are directed nearly at right angles to the primary axis. These branches subsequently proliferate usually three to five secondary branches directed in the same plane or forward. As a result of this continued ramification, the larger cells may be seen to bear at their apices an apparatus consisting of a short axial stalk branching to form a score of ultimate terminations. There can be little doubt that these processes function as absorbing organs and may thus be regarded as haustoria. In stained sections they are often too badly obscured by host protoplasm to be readily distinguishable; but in preparations of material dissected from fresh, living host plants, they may be studied with ease and certainty.

In the meantime the turbinate cell has increased considerably in size and in number of nuclei, the latter usually ranging from 10 to 20 or even more. As no septa have appeared, the parasite is represented at this stage by a simple coenocyte. With the cessation of growth by enlargement, this condition is altered by the appearance of a number of delicate septa, the ultimate number usually ranging from 3 to 5 but occasionally even reaching 7, each of which delimits a peripheral uninucleated mass of protoplasm. As the septa do not appear altogether simultaneously, the first to be inserted represent convex membranes united to the peripheral wall of the turbinate cell along an elliptical line of juncture, the long axis being parallel with the axis of the turbinate cell. The septa inserted later, when the surface of the turbinate cell has been appropriated in considerable measure, are more likely to be in relation to septa previously laid down as well as to the peripheral wall itself. While the protoplasts first delimited thus tend to approach a double-convex, elliptical lenticular shape, the later ones may be more irregular and have several concave facets (Pl. 49, B).

The further development of each of the peripheral protoplasts thus delimited takes place independently of the other protoplasts similarly derived from the same turbinate cell and follows in the main the course described by Maire and Tison (*21*) for *Urophlyctis hemisphaerica* (Speg.) Syd. (*U. kriegeriana* Magnus) and by Vuillemin (*36*) for *U. leproidea*. Material embedded in paraffin, sectioned, and stained shows the protoplasm very slightly contracted away from the septum along the inner surface, and indications of such contraction are present also in freshly dissected material mounted in water (Pl. 48, B, *tb*). This slightly contracted protoplast now pushes out a protuberance from the outer peripheral wall bounding it (Pl. 48, C, D, *tbx*). In those peripheral segments

occupying a position on the side or toward the base of the turbinate cell, the protuberance will invariably take place at some point along the edge closest to the apical end of the turbinate structure; while in the segments on the apical end the protuberance usually occupies a middle position. By the movement of the nucleus and part of the cytoplasm into the protuberance, the tip of the latter becomes somewhat distended. The constricted position now rapidly elongates, resulting in the formation of an attenuated hypha, uniform in thickness and approximately 0.5 μ in diameter (Pl. 48, A–D). The transfer of protoplasm from the peripheral segment to the distended termination continues for some time, until the former has been completely evacuated (Pl. 48, B, D, *ta*).

The elongation of the hypha involves a translatory movement of the termination in a forward direction, from which, however, it may be deflected by a host cell wall, or even reflected back toward the cuticular wall (Pl. 49, B, *tba*). Ultimately elongation ceases, and the terminal distension develops into a turbinate cell entirely similar to the original product of infection, the single nucleus dividing repeatedly to reproduce the coenocytic condition and the branching haustorial process developing from the apical projection, which becomes observable at an early stage during the period of hyphal elongation.

The proliferation of secondary turbinate cells, which tends to be more abundant from the expanded apical end than from regions more nearly basal, thus involves a certain number of lenticular uninucleated masses of protoplasm, always peripheral in position. The larger remaining portion of the contents of the original turbinate cell is consequently not concerned in this process. It may conveniently be designated as the sporogenous cell and always embraces the contents along the longitudinal axis of the spore and as much peripheral protoplasm as is not involved in the peripheral segments. The contents of the sporogenous cell functions in giving rise to a resting spore in the manner described in the following paragraph.

Sooner or later after the segmentation of the turbinate cell has been initiated, the axial haustorial prolongation buds terminally to produce a small globose swelling, which, when it first becomes noticeable, has no demonstrable irregularities on its surface. Later when the swelling or young resting spore has attained a diameter of perhaps 5 μ (Pl. 48, D, *rb*), there are proliferated along a zone midway between the equatorial region and the distil pole from 9 to 15 slender, unbranched, minute processes. The swelling continues to increase in size until it attains the dimensions of the resting spore (about 25 to 35 by 40 to 50 μ), growth in the earlier stages being due mainly to the transfer of protoplasmic contents from the sporogenous cell through the axial haustorial element but later quite largely by the assimilation of food material from the host. Although the surface of the resting spore is rendered impervious by the deposition of a thick wall during the later stages of enlargement, such

assimilation is made possible by the zone of haustorial processes, each of which has in a manner similar to the apical process become branched to form a ramifying apparatus (Pl. 48, A–D, *ra*, *rb*).

DETAILS OF MORPHOLOGY AND CYTOLOGY

The branched haustorial processes with their unusually definite localization, either as a solitary apparatus at the apical end of the vegetative cell or arranged in a well-defined zone between the equator and the distil pole of the resting spore, constitute perhaps the most striking morphological feature of the parasite. Although the literature regarding these structures, especially with reference to their development and orientation on the resting spore, is unsatisfactory, there seems to be good reason to believe that all the other species usually referred to Urophlyctis, as well as many species commonly assigned to related genera, will show complete similarity to *U. alfalfae* in this respect. Thus DeBary (3) in his account of *Physoderma* (*Protomyces*) *menyanthis* states that—

> Auf demselben (distil) Ende der Blasen findet man sehr häufig ein Büschelchen sehr feiner und kurzer in ein Köpfchen endigender Fäden, welche bald verschwinden und über deren Bau und Zweck ich nichts Näheres angeben kann;

and in the figure referred to the appendages are clearly represented at the apices of the obovoid vesicles ("verkerteiformige Blasen"). Lüdi (*15*), who later studied the same fungus, figured a number of unbranched processes arising independently but in close proximity to each other from the apex of the "Sammelzelle"; and in a few cases he represented a hypha arising also independently from the midst of this cluster. Büsgen (*4*) observed the same structure in *Physoderma* (*Cladochytrium*) *butomi* at the apex of the swellings less rich in contents. Like DeBary this author remained uncertain as to their function but considered it probable that the apparatus consists of budding hyphae together with granular host protoplasm. He reported, too, the presence in this species of—

> irregular cylindrical projections which appear early on the spore, and later are not greatly inferior in length to the diameter of the spore. Stained with iodin, a membrane and hyaline contents with a few granules may be recognized. When the spore matures, these break down.

These structures he designated as haustoria and related their function, in our judgement altogether correctly, to the assimilation of food material. His figures, however, with the exception of figure 19, a, which shows a detached branching rhizoid, lack clearness and lead one to believe that probably groups of newly proliferated young turbinate cells were confused with the rhizoids. On the other hand, the haustoria he shows associated with the resting spores of *Physoderma* (*Cladochytrium*) *flammulae* suggest a good possibility of a zonate arrangement similar to that

found in the alfalfa parasite corresponding, for example, to Plate 48, A–D, *ra*, *rb;* although, to be sure, the attachment of the "Sammelzellen" to the convex haustoria-bearing side would be at variance with any close homology. It appears not unreasonable, however, to suspect that Büsgen was in error in regard to this point and that the resting spore may be attached by its concave side, the concavity, as in *Urophlyctis* spp. generally, very probably being opposite the side bearing the haustoria.

Clinton (*5*) noted the presence of a rhizoid-like process on the side of the "Sammelzellen" toward the young sporangium in *Physoderma* (*Cladochytrium*) *maculare* and figured it both as a terminal apical structure before the development of the resting sporangium has been initiated and as a median whorl after the latter has been formed. Regarding its function he states that—

> The exact nature of these processes is not clearly shown, though they seem to bind the sporangium cell to the Sammelzellen.

In his figure 32 he shows a similar process attached to an element that appears to be a young resting spore, although he makes no reference to this condition in the text.

Schroeter (*28*) observed the apical apparatus on the vegetative cells of *Urophlyctis pulposa*, designating it as—

> ein Krönchen, ein Schopf feiner und kurzer, oft verzweigter Protoplasma Anhängsel.

Vuillemin (*35*), who later studied the same species as well as the beet parasite, appears to have recognized the apparatus as consisting of a "tronc" bearing terminally a "houppe" of short ramifying processes—the "panache terminale." To these processes and to the haustoria on the resting spores, as well as to the "appareil nourricier" generally, he (*36*) assigned a structure identical to that of the striated muscle fiber of animals. We have not been able to distinguish anything suggesting striation in any portion of the thallus of *U. alfalfae*. The haustoria here, moreover, appear to have a membrane that seems to persist after the contents have been withdrawn by plasmolysis or have degenerated. The history of the development of the haustoria on the resting spore as given for the beet parasite again is at variance with their development as observed in *U. alfalfae*. For the resting spore, according to Vuillemin, comes about by the swelling of the "sommet du tronc du panache" in such a way that—

> Les branches se trouvent dissociées en plusiers buissons et entraînées à divèrses hauteurs sur la boule terminale, tandis que d'autres fragments sont restés à la base.

Whereas in *U. alfalfae* the resting spore is initiated as a bud from the tip of the axial haustorial element, never involving translocation of any haustorial ramification. And as has been pointed out, the haustoria on the resting spores are subsequently developed as new structures in a well-defined zone and are not portions of the apical haustorium distributed

in a miscellaneous manner over the surface of the resting spore by the enlargement of the latter.

The time of proliferation of the resting spore seems to be rather variable. It may follow immediately after the septum delimiting the last peripheral segment has been laid down, before the proliferation of the new order of turbinate cells has begun (Pl. 48, B, *rb*), or more usually somewhat later when one or more of the peripheral segments have proliferated secondary turbinate cells (Pl. 48, C, *rb*). Or, as is not infrequently the case with the unusually large primary turbinate cells, the immediate product of infection, the resting spores may not be formed until three or four successions have intervened and the original lesion has become a well-developed cavity (Pl. 50, *tba*). The protoplasm in the sporogenous cells of such primary turbinate structures as well as the host protoplasm of cells or cavities that have long harbored the fungus frequently take a dense uniform stain with safranin—a result that might readily be attributed to the diffusion of a deep-staining substance. Where this abnormal condition becomes very pronounced, it is not improbable that no resting spore is produced at all, the deep-staining protoplasm finally disintegrating in place. With perhaps this occasional exception, every turbinate cell produces always one resting spore. According to Maire and Tison (*21*), *Urophlyctis hemisphaerica* (Speg.) Syd. produces first a succession of "vésicules collectives," each of these in turn giving rise to several others of the next order, until ultimately each "vésicule collective" produces only a single resting spore. Such separation of vegetative and reproductive stages is not discernible in *U. alfalfae*, the production of resting spores being common to each order of turbinate cells; and, although toward the end of the season, when conditions for growth become poor, the proliferation of turbinate cells may be considerably reduced, as may be inferred from the relatively small number of young conditions in old galls containing an abundance of mature resting spores, it is questionable whether their production is ever entirely stopped so long as the host tissue is alive and growing.

In this connection it may be mentioned that the presence of unfavorable conditions for development is indicated usually by a very pronounced enlargement of the hyphae. When the parasite is growing vigorously the hyphae, by which the youngest turbinate cells are attached, do not ordinarily exceed 0.5 μ in diameter. Later their diameter ordinarily increases to 0.8 to 1 μ, the increase being, as Vuillemin (*35*) has pointed out, in the wall, the lumen remaining the same and, indeed, soon appearing devoid of protoplasmic contents. In old, overwintering galls, however, there may be found usually an abundance of hyphae measuring 3 to 5 μ, the surface of which may be marked with irregularities which give the structure a granular appearance, especially in stained paraffin sections. Within these hyphae the turbinate cells occur as loculi in distensions occupying junctional or terminal positions and are connected

with each other by the persisting, very narrow, central lumina (Pl. 52, B). Magnus (*20*) designated this as encysted mycelium and regarded it as being probably viable; although the degenerated condition of the protoplasm where this is present, and more particularly the very frequent absence of any contents whatsoever, would not argue for a high degree of vitality. However this may be, the appearance of such swollen mycelium suggests a pathological condition of the parasite rather than a normal one.

Beyond a statement by Wilson (*37*), quite impossible of interpretation in the light of the life history here presented, that the—

> content, cytoplasm, and the nuclei of the resting spores in the dormant condition corresponds to that of the plasmodium in the stage immediately preceding spore formation,

there appear no cytological allusions in the literature on the alfalfa parasite. However, certain details regarding the nuclear behavior in *Urophlyctis rübsaameni* have been given by Bally (*2*), and the valuable paper on *U. hemisphaerica* by Maire and Tison (*21*) contains a brief account of nuclear changes in the congeneric parasite on *Carum incrassatum* and *Kundmannia sicula*.

The variability in size of the nucleus pointed out by these authors is well exemplified also in *Urophlyctis alfalfae*, the larger and smaller dimensions being here generally characteristic of certain stages in the development of the organism. Thus, in the young primary turbinate cell, the nucleus, which is subspherical in shape, commonly measures about 2 μ in diameter and is composed largely of refringent, nonstainable material and a single, very conspicuous, deep-staining body (Pl. 49, A, *ta-tg*). Later, the nuclei may increase appreciably in size, even before their migration into the secondary turbinate cells or into the young spore (Pl. 49, B, *ta*). Considerable increase, however, appears to take place quite invariably in the single nucleus of the young secondary turbinate cell, a maximum diameter of 5 to 6 μ being here attained before division occurs (Pl. 50, *tb-bx*). Division is initiated by the deep-staining body becoming elongated and being drawn out into a spindle-shaped figure, which may be either straight or distinctly cresentic, depending on the curvature of the portion of the nuclear membrane to which it is laterally applied (Pl. 50, *tbd*). This spindle-shaped structure appears to divide in the middle, yielding two bodies similar to the original, which assume positions separated from each other. A membrane is now formed between the two granules, dividing the nonstainable material about equally; and when the two hemispherical division products have rounded up, the structure of the parent nucleus is reestablished, although pairs of sister nuclei can usually be distinguished for some time by their nucleoles facing each other—a figure that is by no means uncommon (Pl. 50, *tab*).

We have never been able to make out in the nucleus at any stage in the development of turbinate cells anything that would need to be inter-

preted as a chromatin network. Occasionally in nearly evacuated sporogenous cells, where the attenuated condition of the cytoplasm permits of more accurate study, strands were observed close to the periphery of the refringent nonstainable portion; however, from their general appearance and staining reaction, it is much more probable that these represent overlying strands of cytoplasm. The chromatin material here seems to be very largely if not completely concentrated in the conspicuous, densely staining body, which may thus be regarded as a karyosome or chromatin-nucleole. This mode of division presumably constitutes a type of amitosis; and, indeed, with a nucleus of the structure described, mitosis of the regular type is manifestly out of question. And yet the enlongated spindle shape assumed by the nucleole suggests that perhaps division here may involve some mechanism resembling in a rudimentary way the apparatus associated with mitosis. The whole process bears considerable resemblance to that described by Kusano (*13*) as occurring in the zoosporangia of *Olpidium viciae*.

By repeated divisions the nuclei in the turbinate cells reach a number of 10 to 20 before the latter has attained its final dimensions; and this increase in number seems to involve usually a decrease in size, which may sometimes be quite insignificant, or again quite considerable, but is nearly always perceptible. Nutrition seems to have some influence on the size of the nuclei at this stage, the turbinate cells found in recently invaded tissues rich in protoplasm generally remaining relatively large throughout, while those farther toward the origin of the cavity appear to suffer the greatest reduction.

The cytoplasm of the growing turbinate cells stains moderately deeply and seems to have a uniform, finely granular or reticulate structure. During the earlier stages of growth, a relatively large vacuole may usually be distinguished near the proximal end. Perhaps this is later associated with the insertion of a septum near the base of the cell that is probably not always concerned in delimiting a uninucleated protoplast but appears to serve more frequently in shutting off the protoplasm from the evacuated hypha. Although the number of vacuoles of a size readily to be observed may be increased during the later stages of growth to several, the difference between the basal and distil ends never becomes considerable, the structure of the cytoplasm at the time of the insertion of the peripheral septa being generally rather uniformly granular or finely reticulate. The progressive evacuation of contents of both the peripheral segments and the sporogenous cell brings about an attenuation of the cytoplasm which, especially in the sporogenous cell, is associated with the appearance of large vacuoles that ultimately, with the exception of a few strands of cytoplasm, coalesce to fill the entire cell.

As the isthmuses between the peripheral segments and the anlagen of the young turbinate cells, as well as that between sporogenous cell and resting spore, are considerably narrower than the nuclei, the latter

undergo some distortion in their passage through these communications. The achromatin passes into the lumen of the connecting element as a beaked extension followed by the chromatin-nucleole, which, too, is drawn out in a conspicuous manner (Pl. 49, C). The normal nuclear structure is recovered when the material has reached, for example, the flaring portion of the isthmus at the proximal end of the resting spore. The result of the total protoplasmic movement is that in *Urophlyctis alfalfae* the penultimate cells are either evacuated or in the process of evacuation and that all elements more basal in position, hyphae as well as peripheral segments and sporogenous cells, are always quite empty of living material.

Within the young, growing resting spore, the nuclei increase somewhat in size; but much more marked is the immediate increase in size of the chromatin-nucleoles, which at this stage measure 2 μ in diameter, or approximately half the linear dimensions of the nucleus. It is not improbable that some nuclear divisions may take place. In living material the resting spores show a beautifully vacuolate structure, the vacuoles being numerous and relatively large (Pl. 48, A–D, *ra*, *rb*). This structure is apparently poorly preserved in the processes of killing, embedding, and staining. Microtome sections stained with Flemming's triple combination show the cytoplasm as having a dense reticulate structure readily distinguishable, however, even in the earliest stages from the cytoplasm of the turbinate cells by its greater affinity for gentian violet.

Later, during the maturation period, the cytoplasm of the resting spores appears more loosely reticulate, and the nuclei assume still greater dimensions, finally measuring 6 to 8 μ in diameter (Pl. 49, D–F). This increase in size is associated with the appearance of very minute granules of chromatin more or less irregularly disposed near the periphery of the achromatin mass and easily distinguished from the surrounding cytoplasm by a marked difference in staining properties. In many cases the arrangement in a definite reticulum is particularly pronounced (Pl. 49, F). Maire and Tison (*21*) report that in the resting spore of *Urophlyctis hemisphaerica* certain nuclei become enlarged, their nucleoles becoming vacuolated and giving rise to large masses of a substance staining red with safranin which accumulate in the center of the spore. Something similar seems to occur in the maturing resting spores of *U alfalfae*. Plate 49, F, represents an early stage in the process, the three nuclei shown in the center having become conspicuously enlarged, the achromatin having partly lost its refringency, and the nuclear contours having become less distinct. Later, as in Plate 49, E, the chromatin masses are no longer distinguishable but appear to have been transformed or replaced by vacuolate cytoplasm somewhat more attenuated than at the periphery and inclosing in its meshes the numerous granules of red-staining material that have presumably been derived

from the chromatin. Plate 49, D, shows a condition that frequently appears in spores that probably have been poorly nourished. The degeneration of the central nuclei leads to the origin of a large vacuole that ultimately develops into a cavity near the periphery of which a variable number of red-staining granules are always to be found.

Maturation involves, too, a conspicuous transformation and thickening of the wall of the resting spore. Even while growth is still proceeding, the spore wall becomes increasingly thick; and during the later stages of enlargement, although still capable of further distension, in all probability it no longer permits of an easy passage of food materials. After final size is attained, thickening proceeds rapidly. The mature spore wall is a structure about 1.5 μ in thickness, of a yellow, vitreous appearance, inelastic and brittle; when the wall is fractured by pressure applied in manipulation, fragments may break out like pieces of shell from a nut, often leaving the contents quite intact.

When the spore has attained maturity, the haustorial processes disappear, whether by retraction, degeneration, abscission, or accidental fracture could not be definitely determined. However this may be, a circle of pits or scars, corresponding in number and position to the haustoria (Pl. 48, F, G), is always left, because the thickening of the spore wall never involves the places of attachment of the haustoria. In examinations of herbarium material, in which turbinate cells and hyphae are only too frequently quite unrecognizable, these pits serve as a morphological feature of no mean taxonomic value.

GENERAL TAXONOMIC CONSIDERATIONS

The taxonomic relations of the plants included under the genera Urophlyctis, Physoderma, and Cladochytrium remain in need of study. Schroeter (*29*) saw in the association of the "Oosporangium" of *U. pulposa* with the "leere Blase" a sexual apparatus consisting of two conjugating "Fruchtkörper," one of which has yielded its contents to the other. On the basis of this interpretation he erected the genus Urophlyctis, including it with Diplophysa and Polyphagus in the Oochytriaceae, which family he distinguished from all the other families in the Chytridineae not excluding the Cladochtriaceae, under which were brought Physoderma and Cladochytrium by the presence of sexuality in the origin of the resting spores. Fischer (*9*), on the other hand, denied the existence of sexuality in Schroeter's genus and placed it with Physoderma as a subgenus under Cladochytrium. Schroeter's views received support from Magnus, who described a number of forms—*U. kriegeriana* (*18*), *U. leproidea* (*18*), *U. rübsaameni* (*19*), and *U. alfalfae* (*20*)—as congeneric with *U. pulposa* and exhibiting the same type of oogamy. The later investigations on *U. leproidea* by Vuillemin (*35*), on *U. rübsaameni* by Bally (*2*), and on *U. hemisphaerica* by Maire and Tison (*21*) have

not confirmed Magnus' assumption of sexuality in these forms; and from the present account it is obvious that in the formation of the resting spores of *U. alfalfae* there is no indication of any process of conjugation.

In order to determine more nearly in what measure the development and morphology of the alfalfa parasite might be common to related forms, the writers examined herbarium material of various species of Urophlyctis, Physoderma, and Cladochytrium. Fresh living material of a species other than *U. alfalfae* was obtained only from *U. pluriannulatus* (B. and C.) Farlow (7), occurring in the Pacific States on *Sanicula menziesii*, on which host it was collected in excellent condition near Philomath, Oreg., on April 7 and May 16, 1919. As its range extends over the region in which crownwart is known, suspicion has arisen now and then that the two parasites might be identical. This suspicion may now be definitely dismissed.

Urophlyctis pluriannulatus may very easily be dissected from the cavities in the wartlike protuberances on the stems and leaves of diseased plants of *Sanicula menziesii* (Pl. 53). Mounts of thalli consisting of hundreds of turbinate cells and resting spores in a good state of preservation were obtained in this way. Plate 52, A, C, shows two small portions of such a thallus. The general method of development corresponds exactly to that described for *U. alfalfae*, yet morphological differences sufficient to separate the two as distinct species are readily recognizable. Greater dimensions are characteristic of *U. pluriannulatus*, both of turbinate cells (which measure approximately 22 μ in length and 18 μ in major diameter, against 19 μ length and 15 μ major diameter for *U. alfalfae*), and of resting spores, the equatorial diameter here ranging from 45 to 60 μ, as contrasted with 40 to 50 μ for *U. alfalfae*. The turbinate cells of *U. alfalfae* produce usually a maximum of four to five secondary turbinate cells, a greater number being occasionally produced, however, by the very large primary turbinate structures; whereas in *U. pluriannulatus*, turbinate cells not infrequently produce seven or eight turbinate cells of the next order, five or six being the rule. An interesting but rather inconspicuous difference in the structure of the rhizoids on the resting spores may be noted. Since the primary branches are inserted at nearly right angles in *U. alfalfae* while the corresponding angles tend to be much smaller in *U. pluriannulatus*, there is brought about a difference that might crudely be compared, for example, to the difference in habit between a palm and an elm. In *U. pluriannulatus*, too, the haustoria are inserted slightly nearer the equator than in the alfalfa parasite. But the most unmistakable specific difference is to be found in the number of haustoria on each resting spore, which in *U. alfalfae* varies from 9 to 15 and in *U. pluriannulatus* ranges from 14 to 24. (Compare Pl. 48, E, with Pl. 52, D.)

In this connection it may be mentioned that resting spores from herbarium material of all the other species of Urophlyctis examined, after being

boiled with caustic potash and cleared with chloral hydrate, reveal a ring of pits altogether similar to those observed on spores of *U. alfalfae* and *U. pluriannulatus*. That this implies the presence of haustoria in the following species can hardly be doubted:

Urophlyctis bohemica Bubak on *Trifolium montanum*, Rabenhorst-Pazsche, Fungi Europaei et extraeuropaei, No. 4378.

Urophlyctis kriegeriana Mag. on *Carum carvi*, Jaap. Fungi sel. exs. No. 126.

Urophlyctis kriegeriana Mag. on *Pimpinella nigra*, Bubak, F. Fungi Bohemici June 9, 1901.

Urophlyctis magnusiana Neger on *Odontites rubra*, Vestergreen, Mic. rar. sel. No. 1614.

Urophlyctis major Schroeter on *Rumex britannica*, Davis, J. J., Wisconsin fungi. Aug. 27, 1913.

Urophlyctis pulposa (Wallr.) Schroeter on *Chenopodium glaucum*, Sydow Myc. ger. No. 1086.

Urophlyctis rübsaameni Magnus on *Rumex scutatus*, Jaap, O Fungi sel. exs. No. 402.

Seventeen species of Physoderma and Cladochytrium were also examined by the same method, and of these at least 2 species—namely, *Physoderma menthae* Schroeter on *Mentha aquatica*, Vestergreen, Mic. rar. sel. No. 1609, and *P. zeae-maydis* on *Zea mays*, material furnished by W. H. Tisdale—revealed a zone of pits, although no direct evidence could be obtained that these had served as places of attachment for haustoria. It is interesting to note that a certain range in number of pits was found to be characteristic of species and that even numbers seemed to predominate. Thus *Urophlyctis rübsaameni* showed either 6 or 8. Pronounced and constant disparity in number of pits may, indeed, be interpreted as indicating rather clearly that forms assigned to the same species because of close relationship of their hosts may belong to quite different species. It appears hardly admissible, for example, to designate the parasite on *Pimpinella nigra* with 10 to 14 pits as *U. kriegeriana*, when this species of *Carum carvi* shows only from 6 to 10; and the identity of *U. kriegeriana* and *U. pluriannulatus*, suggested by Farlow (7) as a fair possibility, would seem to be equally improbable.

In a number of species as, for example, *Physoderma maculare* (5), *P. butmio* (4), and *P. zeae-maydis* (33), the germination of the resting sporangium involves the lifting off of a circumscribed portion of the spore wall by the expanding endosporangium. Although this "lid" is usually not apparent in the spore wall, its presence on the resting spores of *P. comari*, *P. cleochardis*, *P. gerhardti*, *P. iridis*, *P. menthae*, *P. schroeteri*, *P. vagans*, and *P. graminis* could be determined from an examination of herbarium material with moderate certainty. It remains a question whether the resting spores of those species in which nothing resembling a lid could be made out, including for example, *P. agrostidis*,

P. calami, *P. hipuridis*, *P. spargani*, and *P. speciosum*, germinate, perhaps, in a manner similar to *P. menyanthis*, in which, according to Clinton (*5*), the outer wall is ruptured by the elongating protoplast, dehiscence of the zoospores taking place at the tip of the protrusion. The absence of any indication of lids from the spores of all species of Urophlyctis examined may be of taxonomic significance, although this can not be determined until more reliable results have been obtained in the germination of the spores. It would be interesting, too, to determine from living material the positional relation between the zone of haustoria and the lid in those species where both appear to be present, as seems to be the case, for example, in *P. menthae* and *P. zeae-maydis*.

The more striking recorded departures of a number of species of Physoderma from the general thallus structure of the two species of Urophlyctis investigated by us remain in need of explanation. One of the departures is found in the septation of turbinate cells and in the fate of the different segments. As has been pointed out, in *Urophlyctis alfalfae* and *U. pluriannulatus* the production of secondary turbinate cells always starts with the delimitation of peripheral segments that involve portions of the parent cell wall, most frequently subapical or lateral and occasionally subbasal. The distinction between a smaller basal cell and a larger distil cell, made by Büsgen for *Physoderma butomi* (*3*) and by Clinton (*5*) for *P. maculare*, is thus without significance here; while their accounts of the origin of the resting spore from the proximal cell are directly at variance with developments in *U. alfalfae* and *U. pluriannulatus*, in which the resting spore is invariably developed from the large multinucleate residue not involved in peripheral segments. Lüdi (*15*) figured the "Sammelzellen" of *P. menyanthis* with 1 or 2 transverse septa and represented the resting spore as being attached to the distil segment thus delimited by a filament of considerable length. According to this writer's account, the resting spore here is not always terminal, but by itself proliferating a "Sammelzelle" it often appears as an intercalary structure associated with two "Sammelzellen." Tisdale's (*33*) account of *P. zeae-maydis* presents even more points of difference, showing structures consisting of two to four lobulate segments set off by transverse septa, these segments, with the exception of one, capable of forming a resting spore either directly or at the end of a fiber. In this form, organization and development would appear to be of a rather miscellaneous type, contrasting sharply with the definite sequence of growth found in the two plants figured in this paper.

Reference has been made elsewhere to Büsgen's figures of *Physoderma* (*Cladochytrium*) *flammulae*, in which the resting spore is represented as being attached to the "Sammelzelle" by the side bearing the haustoria. Another detail worthy of note in the same figure of Büsgen's is the length of the hypha connecting "Sammelzelle" and resting spore, approximating as it does half the length of the resting spore. In Cornu's

(*6*) figures of *P. maculare* (*Melanotaenium alismatis*), the hypha connecting "corps central" and spore is even longer, exceeding here the length of the "corps central"; and, as has been indicated above, an entirely comparable figure is given by Lüdi to illustrate conditions in *P. menyanthis*. If these writers have not mistaken turbinate cells (or their homologues) for resting spores and have not erred in relating the latter to the wrong turbinate cells, it would appear that conspicuous variability in length is characteristic of the connecting isthmus which in *Urophlyctis alfalfae* and *U. pluriannulatus* is extremely short.

Magnus emphasized the difference in anatomical effects produced by species he referred to the genus Urophlyctis and by those he assigned to Physoderma. The former cause hypertrophy and thickening of host cell wall, while the latter leave the host tissue in an approximately normal condition. Perhaps a distinction on such grounds would make the classification of parasitic forms contingent in too large a measure on reactions of the host plant to be admissible in a taxonomic sense. It seems not improbable that further study of the plants now referred to Urophlyctis, Physoderma, Cladochytrium, and perhaps a few other related genera will reveal possibilities in generic regrouping based on the more significant similarities and differences in morphology and development.

PATHOLOGICAL MORPHOLOGY

It has already been stated that the fungus attacks primarily leaf scales and leaves at a very early stage of development in the growing bud. Only rarely has it been found to have penetrated to the axis in the dividing undifferentiated tissue of the bud. The stimulative effect of the fungus is limited strictly to the structure which has been invaded, while other structures in the vicinity of the main axis and the axis itself show retardation and often cessation of development.

The first morphological change in the host consequent upon invasion consists in a slight enlargement of the first cell entered so that it comes to project both outwardly and inwardly against the underlying cells. These underlying cells may also show a slight enlargement before they are actually entered by the advancing fungus. The nuclei of the affected cells enlarge notably, and the large deep-staining nucleoles persist for a long time in the fungus cavities, their number serving as an index to the number of host cells that have been destroyed.

The fungus evidently gains access to new cells by the solution of thin cell walls in advance of the growing turbinate cells. In early development when a number of these fungus cells are advancing close together in the same direction, the walls of the host cells are found dissolved before the fungus comes in contact with them (Pl. 55), thus precluding the possibility of mechanical pressure as a factor in effecting the advance. In later stages, however, when turbinate cells are fewer and more scattered,

the host wall does not always yield until the advancing cell is in contact with it, suggesting that mechanical pressure may here be a factor.

The enlargement of cells under the stimulus of the fungus is the smaller factor in the production of galls. As soon as the fungus has begun its advance into the tissue, cell division is stimulated in the vicinity, and even at a considerable distance if the fungus is making rapid growth. The first notable divisions take place in the cells just beneath the epidermis in the region of the point of invasion (Pl. 55). Walls are inserted tangentially to the outer surface of the structure, and the increase in tissue at this point surrounds and may even bury deeply the base of the fungus cavity so that it no longer leads to the exterior of the gall. The thin-walled parenchyma in which the fungus forms its cavities may show little morphological change near the invader in the early stages of its progress, especially if these cells have matured and are not readily capable of division. However, the older part of the surrounding wall of the fungus cavity is soon greatly thickened with a layer which is very brittle when cut and which is therefore poorly preserved in stained preparations. The peculiar structure and markings sometimes found in these walls has been noted by Magnus (*20*), though his assumption that the window-like openings between fungus cavities are due to the local absorption of these walls seems less probable than that they are the partly filled openings through which the fungus advanced at an earlier stage. As soon as this thickening is well under way, the host cells adjoining the cavity begin to divide with walls tending to be oriented tangentially to the wall of the cavity. Such divisions proceed further in the vicinity of vascular bundles than elsewhere, giving rise to a considerable mass of cells in parallel rows, almost cubical in shape, with walls a little thicker than those of the normal parenchyma (Pl. 56, A). But these processes are rarely rapid enough to surround the newer portions of the cavity where the fungus is slowly breaking into cells and extending its ramifying maze. Perhaps the larger bulk of the cells that make up the gall are developed from the vascular bundles where division, especially in later stages in development, becomes very active. Sometimes a bundle becomes much broadened, and from the active cambial region a large mass of parenchyma on one side and a few leaf tracheids on the other are set off. Tissue from this source is likely to be richer in protoplasmic contents than that from the other sources mentioned and is more extensively penetrated by the advancing fungus. Thus, it may be said that the response of the cells to the stimulation of the fungus is in proportion to their capability for meristematic activity and to their nearness to the source of stimulation. Cells near the exterior of the gall divide with walls tangential to the surface of the gall; those in close proximity to the older portions of the fungus cavity divide with walls tangential to the wall of the cavity; while vascular bundles function in division

like stem bundles in giving rise to secondary thickening, producing irregular masses of leaf elements. Thus, the normal limitation in the direction of cell division and growth which produces thin, laminated structures is removed, and thick, fleshy amorphous masses of tissue inclosing ramifying cavities filled with the fungus in all stages of development are produced. On irrigated land these structures are not usually well protected by epidermis or cortex and readily dry out or decay, but in dry regions many become covered with a corky layer that protects them from destruction.

In partial contrast to the galls upon alfalfa is the gall upon *Sanicula menziesii* (Pl. 53) caused by *Urophlyctis pluriannulatus* previously mentioned, a contrast indicated by Magnus (*19*) in his classification of Urophlyctis galls into two types, those upon underground parts of plants and those upon aerial parts. Although the earliest stages in the formation of these galls have not been traced, evidence from more mature stages indicates that the general development is similar to that of galls formed on alfalfa and in fact is exactly like that of the blister-like galls sometimes found on alfalfa leaves. In the attack of the fungus on Sanicula, infection of the leaf, petiole, and stem structures takes place at a later stage of host development than is common on alfalfa, and the response of the host tissue to the stimulus of the fungus is not nearly so great, extending only to a distance of a few cells. Apparently a small number of cells are rapidly invaded soon after the fungus enters the host. Thickening of the host cell walls around the cavity formed, especially its basal portion, soon occurs; and thereafter it appears that a part at least of the enlargement of the fungus cavity is accomplished by the pressure of the growing fungus mass against the surrounding cells, which become flattened and distorted. Thus, each infection produces one partly chambered cavity in the parenchymatous tissue which has become hypertrophied to form a small blister-like gall.

INOCULATION EXPERIMENTS

In order to avert any possible danger of spread of the disease from experimental plots, inoculation experiments were limited to a few potted plants in a greenhouse at Washington and to plants in the greenhouse and on the trial grounds of the United States Plant Introduction Garden at Chico, Calif. At the latter place, perhaps because of the limited time during which work was done there, no success was attained in producing infection. Since one of these failures may be significant, it will be mentioned. On April 15, 1918, nine days after wart was first found developing on plants in the field, an inoculum was prepared by shaking soil and the fragments of decomposed warts from the crowns of a large number of plants which had been badly diseased the previous year and adding a small amount of crushed warts which had been found not yet decayed.

A square yard of vigorously growing alfalfa plants in the corner of a 2-year-old plot was selected for inoculation. These plants were already producing shoots 1 foot or more in height. The soil and débris were carefully scraped away from around the crowns of these plants, exposing a large number of developing buds and shoots. The inoculum was carefully packed around these crowns, the growing tops of which were finally sprinkled and dusted with crushed galls. Sphagnum was packed over and around the plants to a depth of 2 or 3 inches, water was sprayed over the plot, and the sphagnum and soil beneath were kept thoroughly wet for 10 days. On June 1 the material was removed from around the plants, but no trace of any infection was discovered. Whether the rapid growth which the plants were already making at the time when inoculation was made prevented infection or whether some other circumstance was responsible for the failure can not be told until further work is done. From observations which were made in the field, it appears probable that most of the warts which developed that spring resulted from infections which had taken place previous to the date at which the inoculation was made. Thus it is possible that at the late date at which the experiment was begun the spores of the fungus had in large part ceased to germinate, or the plant itself might have passed its period of greatest susceptibility.

Inoculations of plants in the greenhouse at Washington gave two instances of successful infection. In one case a pot of seedling plants about 6 inches tall were inoculated by replacing the dirt around the crowns with crushed diseased tissue and débris from plants recently received from California. Inoculation was made October 1, and on January 3 three plants with very young infections were found.

Attempts to obtain infected plants by sowing seed in soil to which crushed warts had been added usually resulted in the destruction of the young plants by Rhizoctonia and possibly other fungi introduced with the inoculum. In one case, however, among nine plants from seed mixed with Urophlyctis spores and sown in April there were found in the following January three infected plants, two of which were dwarfed and much injured by the disease. If it were possible to obtain a large percentage of plants in the field as badly infected as those in this experiment, this disease would be capable of much harm. As a matter of fact, however, only a relatively small percentage of young plants have been found infected in the field even under what would appear to be the most favorable conditions.

When germination of spores can be obtained with some degree of certainty or when field experiments under suitably controlled conditions can be freely undertaken, opportunity will be open for further infection studies that should add to our meager knowledge of the conditions necessary for infection in the field.

CLIMATE IN RELATION TO THE DISEASE

The fact that the disease has apparently remained so long limited in its distribution to certain regions in the western portion of the country without invading the larger alfalfa-growing areas in the central portion of the country raises the question whether this limitation is due to certain climatic conditions which favor the development of the fungus in these localities or to some factors which have prevented the spread of the causal organism. That the spread of the organism has been inhibited by lack of facilities for distribution is hard to imagine. Even if it should be found that the spores are incapable of withstanding the drying incident to being transported with seed or hay, still a considerable number of plants have been and still are transported by individuals for trial or experimental purposes, and it is hard to believe that no warted plants have been sent at some time into the central and eastern States. On the other hand, it is not easy to discover any common factors of climate in the regions where the disease now occurs which do not exist in the larger eastern regions. For the most part, the disease exists in valleys where the winter is very mild and where there is at least a slight growth of the plant during every month of the year. Such conditions would seem to furnish a long period favorable for infection. However, the disease also occurs in the Salt Lake Valley in Utah and in certain high mountain valleys where the winter is severe. The mere fact of severe winter does not seem to be the sole limiting factor. Thus, it is not possible to answer with an opinion based upon suitable evidence the most important question from an economic point of view that is being asked regarding the disease. Of course it might be determined decisively whether the disease can develop in the central and eastern portions of the country by bringing diseased plants into these regions and observing their behavior. Fear that such experiments might result in a destructive spread of the disease has prevented the initiation of such experiments thus far.

CONTROL MEASURES

Thus far no experimental work bearing directly upon control measures has been undertaken. The direction which such experimental work should take appears to be clearly indicated by the observation of the field conditions under which the disease now becomes most abundant. The one condition which more than any other appears to favor the development of the disease is an excess of moisture in the soil in the early spring when it appears that infection must take place if at all. Any measure which will avert this excess, as by drainage or a diminished supply of irrigation water, should bring about a reduction in the amount of disease.

Under some conditions deep cultivation may reduce the disease. In the spring of 1918 some fields which had received a thorough and deep

cultivation in February were observed to have less of the disease than neighboring fields which had not been so treated. There was ample evidence that the disease had been severe in these fields in the previous season. However, in the following spring the difference between cultivated and uncultivated fields had disappeared.

There is a limited amount of field evidence that the amount of disease is increased when alfalfa is planted directly after alfalfa. Fortunately, such succession is rarely practiced. Thus, on the whole, it can be said that when conditions are made most favorable for the development of the alfalfa plant the disease is diminished, perhaps not so much because the plant is better able to withstand its attacks as because abundant infection is dependent upon conditions which are not of themselves most favorable for plant development.

Search has been made in vain for any evidence of conspicuous cases of apparent resistance to the disease. In one instance in 1919 a plot of alfalfa was found conspicuously freer from the disease than the adjoining plots which appeared to be under exactly the same conditions. It was found that the seed used in this plot was from a different source than that used in the other plots, and in fact the type of plant was different. An effort to obtain seed from this field for experimental work was frustrated by the ravages of grasshoppers. During the following year observation failed to discover any material difference in the amount of disease in this field as compared with its neighbors, and therefore efforts to obtain seed from it were abandoned.

It hardly need be said that until it is known for a certainty whether the disease can be troublesome in the eastern alfalfa-growing regions, care should be taken to prevent its introduction. At least living plants from fields where the disease is known to occur should not be transported to other localities.

SUMMARY

The disease of alfalfa caused by the fungus *Urophlyctis alfalfae*, commonly known as crownwart, has been found to have its origin in the infection of very young buds, the foliar elements of which develop into abnormalities not involving the mature structures of root or stem.

Infection appears to take place only early in the spring, becoming easily discoverable in the latter part of March or in early April in northern California.

In irrigated regions, or in regions where there is abundant moisture during the entire season, most of the galls reach full development early in the summer and thereafter decay rapidly, only a few surviving until the next spring.

The thallus of the fungus consists of two types of structures, turbinate cells and resting spores. In the first turbinate cell that is the immediate development of the infecting fungus are inserted a number of septa

which delimit uninucleated peripheral segments from a polynucleated central sporogenous mass. A hyphal structure of limited growth develops from each of these segments and carries the nucleus in its expanded termination, the latter constituting a young turbinate cell of the next succession. At its mature stage the turbinate cell bears a branched apical haustorium, the short axial element of which proliferates at its tip a globose terminal expansion into which the polynucleate sporogenous mass of protoplasm migrates to produce the resting spore. This is characterized by 9 to 15 branched haustoria in zonate arrangement between the equator and the distal pole.

A solution of the thinner cell walls in proximity to the young, advancing turbinate cells leads to the development of cavities in the hypertrophied tissue in which the resting spores are finally found inclosed.

The abundant development of the disease in the regions where it now occurs is apparently associated with excessive moisture during the period when infection is taking place. Any measures which can be taken to reduce the moisture near the surface of the soil at this time should reduce the disease.

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PLATE 47

Urophlyctis alfalfae:

Drawing of alfalfa plant, showing abundance of crownwart, as found early in May, 1919, in northern California. The dotted line *gl* represents the ground level. Varying degrees of abnormality in the development of the buds are shown. × ⅜.


gl
gl
C Drechsler del

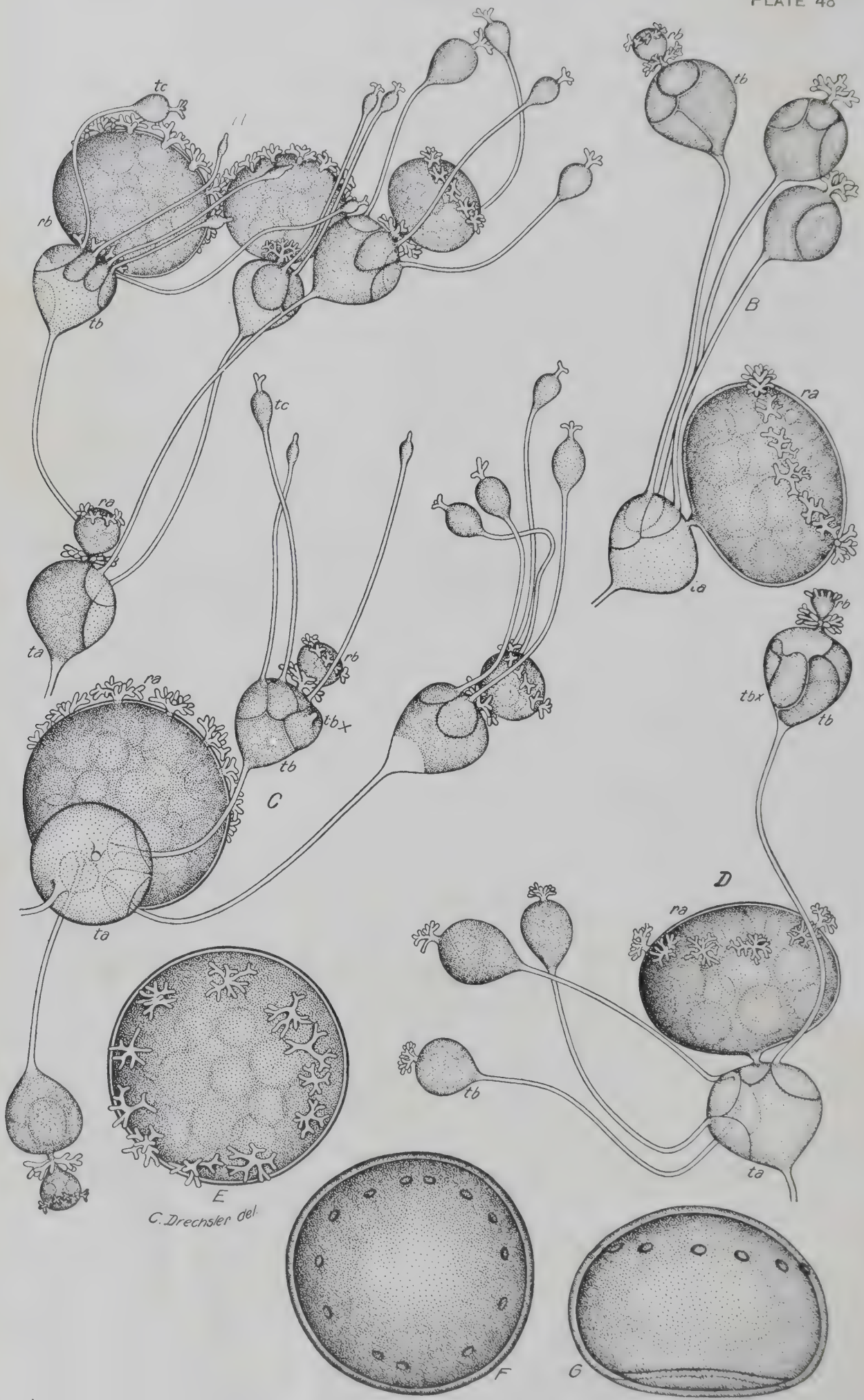

tc
rb
tb
ra
ta
B
ra
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tc
rb
tbx
tb
C
b
ta
rb
tbx
tb
D
ra
tb
ta
E
C. Drechsler del.
F
G

PLATE 48

Urophlyctis alfalfae:

A–D.—Peripheral portions of actively growing thallus of parasite dissected from living host: *ta*, *tb*, *tc*, turbinate cells of successive orders; *ra*, *rb*, resting spores produced by successive orders of turbinate cells; *tbx*, peripheral segments beginning to proliferate turbinate cells by budding. Note the single apical haustorium on the developing turbinate cells *tc;* its median position on the isthmus connecting turbinate cell *tb* and developing resting spore *rb;* its absence from isthmus between evacuated turbinate structure B, D, *ta*, and maturing resting spore B, D, *ra*. Note also zonate arrangement of haustoria between equator and distil pole of resting spore, A–D, *ra*, *rbs*.

E.—Nearly mature resting spore viewed from distil side, showing 11 haustoria in zonate arrangement.

F.—Mature resting spore viewed from distil pole, showing 13 pits that mark former location of haustoria.

G.—Mature resting spore viewed in profile, showing pits in zonate arrangement and light concavity on proximal side of spore.

Drawn with the aid of the camera lucida. Approxiately × 847.

PLATE 49

Urophlyctis alfalfae:

A.—Section of epidermal region of young foliar structures, showing young primary turbinate cells *ta-tg*, the first products of infection, within epidermal cells. Note attachment to cuticular wall by attenuated beak, increase in number of fungus nuclei during growth of turbinate cells, and pathological enlargement of host nuclei *hn*, in invaded cell, *hnx* being normal host nucleus.

B.—Section of young foliar element, showing wall of invaded epidermal cell disrupted and advance of secondary turbinate cells *tbc-tbe* into underlying tissue. One of the other secondary turbinate cells, *tbb*, is forcing its way down along the host cell wall, while another, *tba*, has been reflected toward the cuticular wall; *ta*, sporogenous cell of primary turbinate structure.

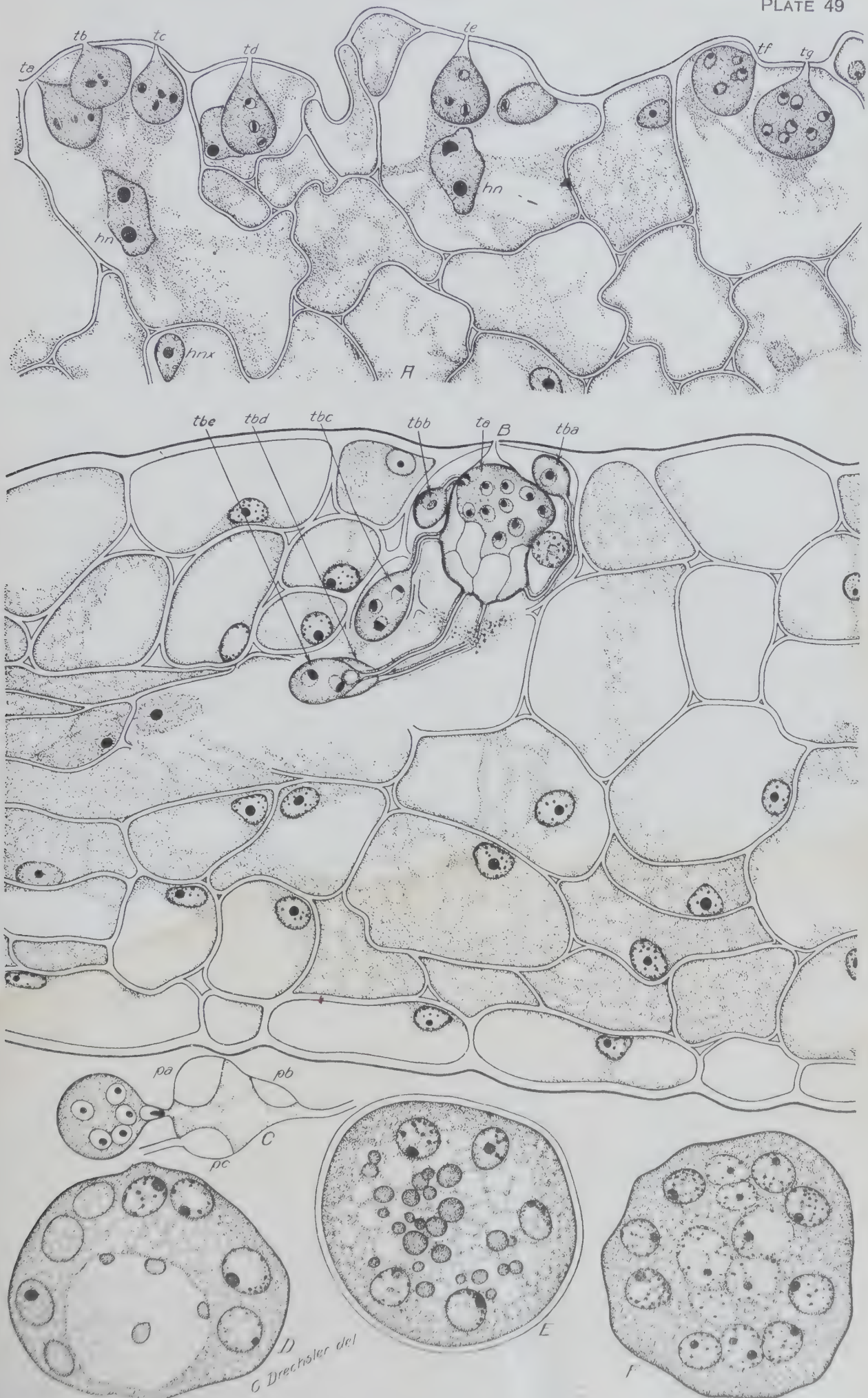
C.—Section of turbinate cell, showing 3 evacuated peripheral segments *pa-pc*. A nucleus is passing through the narrow isthmus connecting the nearly evacuated sporogenous cell with the resting spore, the elongated nucleole following the achromatin.

D.—Section of maturing resting spore, showing 8 nuclei and a central vacuole containing 4 granules staining red.

E.—Section of mature resting spore, showing numerous red-staining granules in center and 5 nuclei.

F.—Section of maturing resting spore, showing 11 normal nuclei and 4 enlarged nuclei in center, the latter apparently degenerating.

Drawn with the aid of the camera lucida. × 860.


ta
tb
tc
td
te
tf
tg
hn
hn
hnx
A
tbe
tbd
tbc
tbb
ta
B
tba
pa
pb
pc
C
D
E
f
G Drechsler del


iba
tc
taa
hn
rs
tbb
tbc
tab
tbbx
tbd
tac

PLATE 50

Urophlyctis alfalfae:

Section of diseased bud scale of alfalfa, showing four coalescing cavities, in three of which the large primary turbinate cells *taa*, *tba*, and *tc* may be distinguished; *taa* has not started to proliferate any resting spore, while the resting spore produced by *tba* is moderately young, although turbinate cells of later orders *tab*, *tbc*, and others have produced resting spores further along in development. The thickening of the host cell walls bounding the cavity and the enlargement of the host nuclei *hn* lying free within the cavity are conspicuous. Note also the large dimensions of the nucleus in the uninucleated turbinate cell *tbbx* and the relatively larger proportions of the nucleoles in the nuclei of the resting spore *rs*. Drawn with the aid of the camera lucida. × 860.

PLATE 51

Urophlyctis alfalfae:

Section of diseased bud scale attacked by *U. alfalfae*, showing a group of eight well-developed cavities *a-h* and their relation to the host tissue. Many of the cells adjacent to the cavities have divided at unusual angles, giving the tissue a characteristic appearance. In *b* the host cytoplasm and fungous material stain unusually deeply, as the result perhaps of general infiltration with some diffusing substance. Drawn with the aid of the camera lucida. Approximately × 417.


a
b
c
d
e
f
g
h
G. Drechsler del


A
tba
rb
tbb
tca
tcb
ra
ta
B
C
D
C Drechsler del

PLATE 52

A, C, D.—*Urophlyctis pluriannulatus*. B.—*Urophlyctis alfalfae*.

A.—Portion of actively growing thallus of *U. pluriannulatus* dissected from gall on leaf of *Sanicula menziesii*, including a turbinate cell *ta* with a nearly mature resting spore *ra; ta* is completely evacuated, having produced 7 turbinate cells of the next order, in one of which *tba* peripheral segments have been delimited, another, *tbb*, has produced two turbinate cells of the tertiary order *tca* and *tcb*, as well as a developing resting spore *rb*. Approximately × 847.

B.—Abnormally enlarged hyphae and turbinate cells of *U. alfalfae*, showing conspicuous thickening of the walls. × 860.

C.—Peripheral portion of actively growing thallus of *U. pluriannulatus*, similar to A, showing 8 turbinate cells of the second order, of which 7 have produced turbinate cells of the last order as well as resting spores. Approximately × 847.

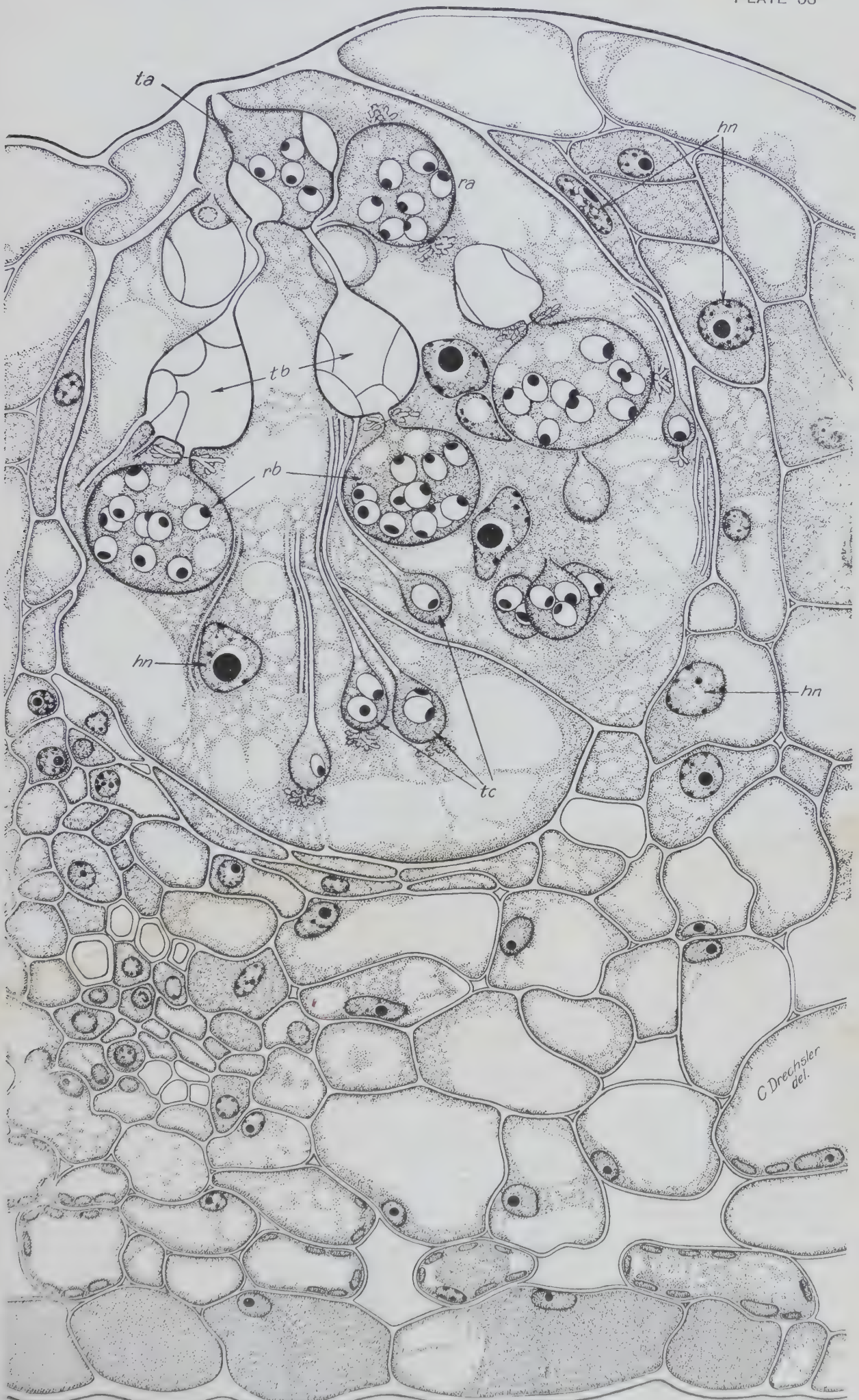
D.—Nearly mature resting spore of *U. pluriannulatus*, viewed from polar end, showing 22 haustoria in zonate arrangement. Approximately × 847.

Drawn with the aid of the camera lucida.

PLATE 53

Urophlyctis pluriannulatus:

Section of leaf of *Sanicula menziesii*, showing development of parasite within gall. Some of the fungus thallus appears to have dropped out of the section in the course of manipulations, as is indicated by the large unoccupied gaps; *ta*, primary turbinate cell; *tb*, *tc*, turbinate structures or cells of successive orders, the former completely evacuated, the latter in early first or second nucleated stage; *ra*, *rb*, resting spores produced by turbinate cells of successive orders; *hn*, host nuclei considerably enlarged as result of influence of parasite. Note the similarity in development of parasite to *U. alfalfae* and the relatively slight influence of parasitism on host anatomy. Drawn with the aid of the camera lucida. × 860.


ta
hn
ra
tb
rb
hn
hn
tc
C. Drechsler del.


A


B

PLATE 54

Crowns of alfalfa plants bearing galls caused by *Urophlyctis alfalfae* photographed at different stages of development.

A.—A comparatively early stage of development at which the origin of the gall structures from the elements of developing buds can be traced.

B.—A later stage of development at which the origin of the tissue has become obscured. The tap root of this crown was cut off and the photograph taken from below. Galls usually become considerably larger than this before they begin to disintegrate if the plant continues vigorous growth.

PLATE 55

A comparatively early stage of host reaction to invasion by *Urophlyctis alfalfae*. The cavities produced by the invading fungus can still be traced from the exterior into the parenchymatous tissue. A few of the cells which are about to be entered by the advancing fungus show some hypertrophy. The division of the cells beneath the epidermis has begun.

Crownwart of Alfalfa Caused by Urophlyctis alfalfae

PLATE 55




A

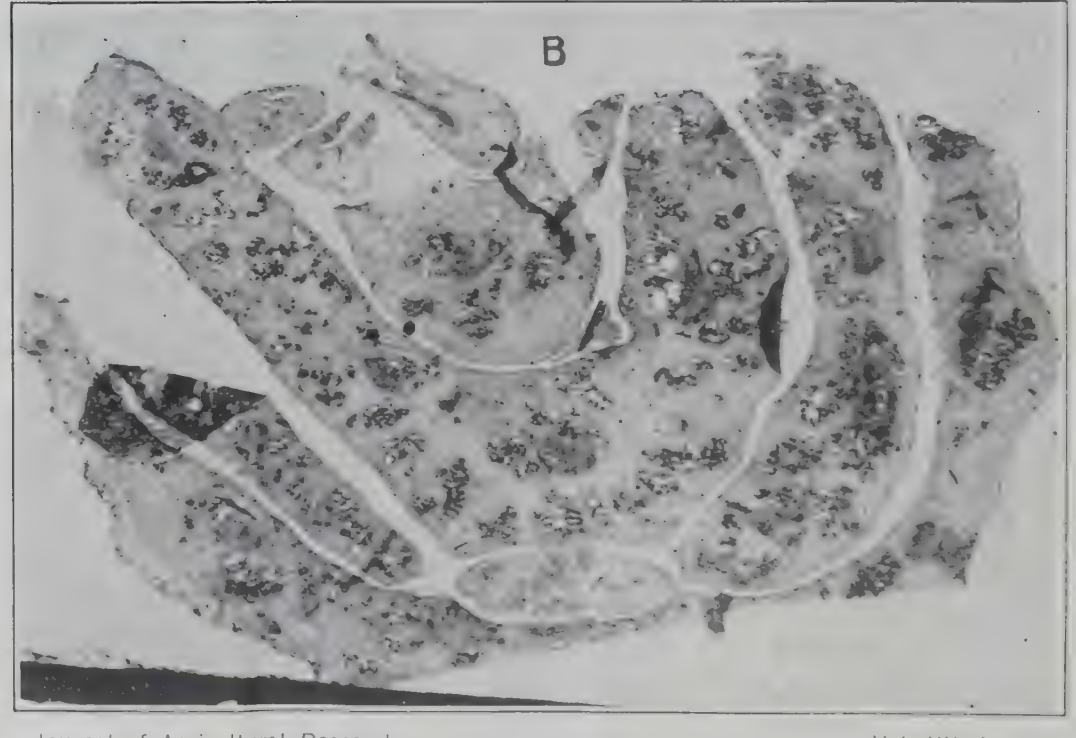

B

PLATE 56

A.—Late stage of development of host reaction to the invasion of *Urophlyctis alfalfae*. Infections have taken place near the tip of a growing point. The division of exterior cells has gone on extensively. The cells around the older portions of the cavity formed by the fungus have also been stimulated to division. The vascular bundle at the center of the mass of tissue has begun to produce parenchyma toward which the fungus is inclined to direct its course.

B.—Vertical section through a well-developed gall near its central axis, showing its laminated structure arising from the thickening of bud elements. The cavities containing the dark-colored spore masses are seen distributed through the tissue, a condition that causes the mottled appearance of the interior of the living gall.

PATHOLOGICAL ANATOMY OF POTATO BLACKLEG

By Ernst F. Artschwager

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Blackleg, as has been shown by the researches of numerous investigators, notably Appel (*2*),[1] Smith (*7*), and Morse (*6*), is a bacterial disease affecting the underground part of the potato stem and the tubers. In its typical form the base of the potato stem shows a pronounced blackening which may extend several inches above the ground. The seed piece from which the diseased plants have been grown is found in a state of decay or is already completely destroyed by rot. The external symptoms are sufficiently striking to enable one to recognize diseased plants even at a distance. Such plants are of a lighter color and usually exhibit a xerophytic texture. They may be normal in size, but most often they are dwarfed and stocky, so that the disease is easily mistaken for leafroll. But while leafroll plants are firmly anchored in the ground, blackleg stems are easily pulled and always show the characteristic lesions on the underground part. The ease with which diseased plants are removed is so striking that one unconciously looks for a contributing mechanical cause, and that such a supposition is not altogether unfounded is shown in a note by Heygi (*4*) who reports wire-worm injuries in almost all the blackleg material that came under this observation. From the results of his investigations, Hegyi is inclined to consider the presence of the bacteria a secondary factor which has nothing to do with the original cause. Yet while it is true that wire worms may cause a loss of many potato hills, they are probably not responsible for the death of plants suffering from blackleg.

The xerophytic texture of the diseased plants is exhibited by stems and leaves alike. The foliage is discolored, usually light and of a metallic luster. The leaflets are folded, and the petiole and midrib are woody and lacking the elasticity and softness which characterize the normal organs. Not all the stems of a diseased plant are necessarily affected. Healthy sprouts may appear side by side with diseased ones (Pl. 57, A), and the diseased sprouts may exhibit various degrees of injury. Plants which have been attacked rather early often continue to live for a considerable period. These plants remain naturally dwarfed, the stalks are spindling, the internodes shortened, and the leaves small. Plants attacked at a more mature age may attain full size, though they usually succumb more quickly to the attack of the parasite than do many of the

[1] Reference is made by number (italic) to "Literature cited," p. 330.

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Key No. G-210

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plants affected earlier in their life. In the last stages of the disease, when the rot has progressed far enough to cut off completely the water supply, the entire plant turns brown and sooner or later, depending on weather conditions, falls prey to the attack of saprophytic bacteria and fungi.

MATERIAL AND METHOD OF STUDY

The field from which the material for study was obtained is located in a clearing of the river bottom land near the Fort Lewis Mesa, Colo. The altitude is 7,500 feet. The soil is a sandy loam containing some organic matter and a water table sufficiently high to insure the growing of a crop without the customary irrigation. The tubers used were of Green Mountain and Rural New Yorker types. They were cut before planting, and because of the apparent soundness of the tubers no surface sterilization was attempted. The season was a normal one. The months of May and June were characterized by excessive dryness. Throughout July and August frequent showers insured a rapid growth of the plants. During the first week of August a severe hailstorm injured the foliage and stems so badly as to make further observations impracticable.

The first diseased plants appeared early in July. Their number increased during the following two weeks and then showed a decline on account of the death of a number of early infected plants and the reduction in number of new infections. Tubers of the same lot which had been disinfected and grown on irrigated mesa soil remained free from disease. The observations made at Fort Lewis were extended on material obtained from other parts of the State, especially the San Louis Valley. In every case the symptoms were similar, the only real difference being in the number of diseased plants per acre.

The plants taken for study were examined while fresh. For the purpose of completing microchemical work and checking results, suitable material was killed in Flemming's weaker solution and embedded in paraffin in the usual way. The principal reagents used were Haidenhein's haematoxylin-safranin stain for histological structures, Devaux's stain for pectic degeneration, phloroglucin-hydrochloric acid for lignification, and Altmann's acid fuchsin stain for protein crystals.

While all previous investigations on the blackleg disease deal with the morphology of the causal organism and its pathogenicity, this study has for its object a consideration of the pathological changes concomitant to the presence of the organism.

PATHOLOGICAL ANATOMY

In general, the histological changes consist in an increase of strongly lignified vascular tissue and in a transformation of part or most of the parenchyma cells of pith and cortex into sclereids (Pl. 58, B). Cytological abnormalities lie mainly in the occurrence of protein crystals in the parenchyma cells of the leaves, the stems, and the tubers.

The elements of the xylem are normal in size, though occasionally they appear smaller. The lumen is reduced; the walls are thicker and more strongly lignified. Even in unstained sections and without the microscope the xylem appears to be darkened. The discoloration often extends to the stem apex and into the petiole, but it is most pronounced in the underground parts of the stem where the external symptoms are most striking. Usually the cell wall alone is discolored, but sometimes a brown, gummy deposit is found in the lumen of the cells, especially of the larger vessels. In typical cases, only the primary xylem is affected; in advanced stages, however, a part of the secondary xylem may also show the browning of the walls. This discoloration of the elements of the xylem is not necessarily a symptom limited to blackleg, since it is associated with numerous other pathological disturbances and is commonly observed in plants which are suffering from excess of water.

The phloem fibers are more abundant. They, too, show a general increase in wall thickening and intensity of lignification. The secondary wall often is so thick as completely to fill the lumen (Pl. 57, C; 58, A); it is distinctly layered and contains numerous simple pits.

While one occasionally finds sclereids in the cortex of the underground stem of the normal plant, there is nothing that could compare with their relative abundance in plants suffering from blackleg. These sclereids are typical parenchyma cells with strongly lignified secondary walls (Pl. 57, C; 58, A). They are either scattered or form solid masses of tissue, often completely replacing the pith and part of the cortex. The transformation of pith cells into sclereids is most pronounced in the apical stem region and in the petiole. In the midrib and in the stem region close to the base, where the browning of the xylem is most pronounced, relatively few sclereids are found.

In the small parenchyma cells of the perimedullary zone similar changes occur. The cells show at first pectic degeneration, which is followed by lignification. The peripheral pith cells, especially in the interfascicular region, are sometimes completely transformed so that they form a sclerenchymatous sheath on the inner face of the vascular tissue.

The phloem elements are mostly normal at the base of the stem but show increasingly advanced pathological changes toward the apex and in the petiole. The cell walls are swollen, occasionally necrotic. The cells of the pericycle undergo similar changes which are more severe and are noticeable even in the lower stem regions.

Plants which are infected early but do not succumb to the attack of the parasite very readily show the most typical and pronounced symptoms. In plants in which the course of the disease has been a rapid one, relatively few changes are exhibited. It will be understood, however, that individual plants vary and that the environment, the age of the plant, and its physiological constitution will in a large measure determine the degree of anatomical changes in tissues and organs.

The presence and activity of the blackleg organism results in a gradual or rapid cutting off of the water supply from the roots and in a break in the path of translocation for plastic materials in the lower stem region. As a consequence of the decreased water supply, we have a decrease in growth activities, especially a check in elongation. The newly formed cells seem to mature more rapidly; in fact, mature and already strongly lignified cells are found close to the growing apex. As long as the leaves remain green and a minimum of water is insured synthesis of foods will go on, though less extensively than in healthy plants. There is not, however, an accumulation of starch as is commonly found in plants suffering from leafroll, but there is a utilization of the food in the laying down of extensive secondary thickenings in the cells of the xylem and fibers and in a transformation of parenchyma cells into thick-walled sclereids. Morse (*6*) reports that when the progress of the disease is slow—

> numerous aerial tubers will be formed on the stalks at the surface of the ground or in the axils of the leaves above.

It would be of interest to know whether in such a case the same anatomical changes occur which normally accompany blackleg.

Just as the formation of sclereids is the most pronounced histological symptom, the appearance of protein crystals in all organs of the plant, the leaves in particular, is a cytological phenomenon always associated with the disease. Protein crystals have been found in the tubers of normal plants. Bailey (*1*) reports the occurrence of cubical crystals in the tubers of *Solanum tuberosum*. A few years later Cohn (*3*) by the use of protein reactions identified the crystals of Bailey as belonging to the typical group. Heinricher (*5*) observed that in potato plants where the root system had been destroyed by decay the basal portions of the plant contained cubical protein crystals which were especially abundant in the cells of the phloem but were altogether absent from the cells of the epidermis and the collenchyma. Crystals have not been found in the aerial parts of the normal plant, and in the researches of the writer on the anatomy of the potato plant and the pathological anatomy of the leafroll disease they have not been observed. However, crystals have been noted by Stock (*8*) in aerial, axillary tubers, where they show the same distribution in peripheral cells of the cortex as do normally developed underground tubers. Protein crystals occur in great abundance in all organs of "blackleg" plants, especially in the leaves (Pl. 57, B; fig. 1). The crystals are usually cubical and vary in size from minute bodies to large structures with a diameter of two-thirds the size of a palisade cell. They are normally found in the cell sap or in the cytoplasm, very rarely inside the nucleus, although nuclear crystals, according to the extensive researches of Zimmermann (*9*), are not at all uncommon.

Nothing definite may be said in regard to the physiological importance of these structures. Crystals have been observed in many plants, in the fungi as well as the highly specialized angiosperms; but, while certain groups of plants show them in great abundance, other plant groups show just as conspicuous a lack. Heinricher (*5*) believed that the interception of the movement of plastic material to the roots causes a forcible deposition of the protein in the basal parts of the stem. This, however, could in itself not account for their formation as has already been pointed out by Stock (*8*), who observed protein crystals in aerial tubers but failed to find them in the stem, although the cells in the latter are completely filled with starch. The crystals probably constitute transitory food which may be used again in the metabolism of the plant and may accumulate when growth is inhibited unless an excess of photosynthetic products (as starch in the case of leafroll plants) stops protein synthesis altogether.

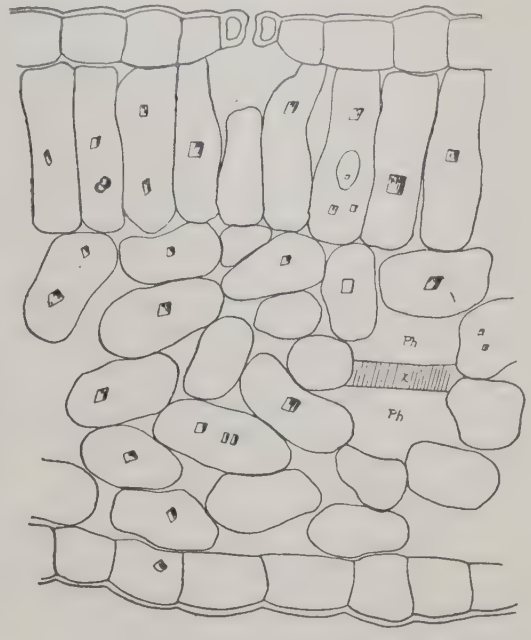


FIG. 1.—Section of potato leaf, showing distribution of protein crystals.

SUMMARY

(1) Potato plants affected with blackleg show an increase in strongly lignified vascular tissue and a transformation of part or most of the parenchyma cells of cortex and pith into sclereids.

(2) Associated with blackleg is the occurrence of protein crystals, especially in the cells of the leaves. Under normal conditions protein crystals have been observed only in the peripheral cell layers of the cortex of the potato tubers.

(3) Only diseased plants grown in the arid western parts of Colorado have been studied. It is possible that plants grown in the eastern United States and at a lower altitude do not exhibit the anatomical changes reported in this paper.

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PLATE 57

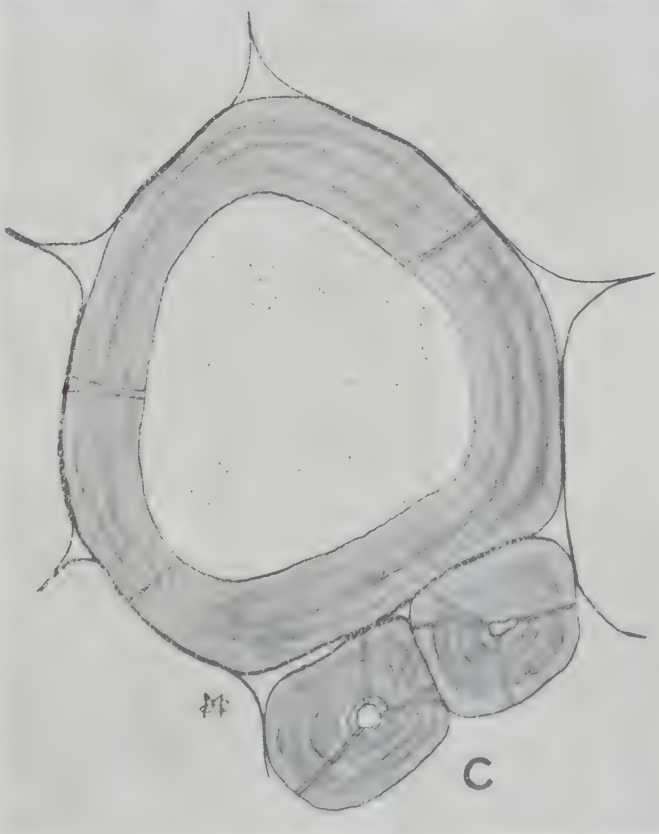
A.—Plant affected with blackleg. One stem is healthy, while the other is severely diseased.

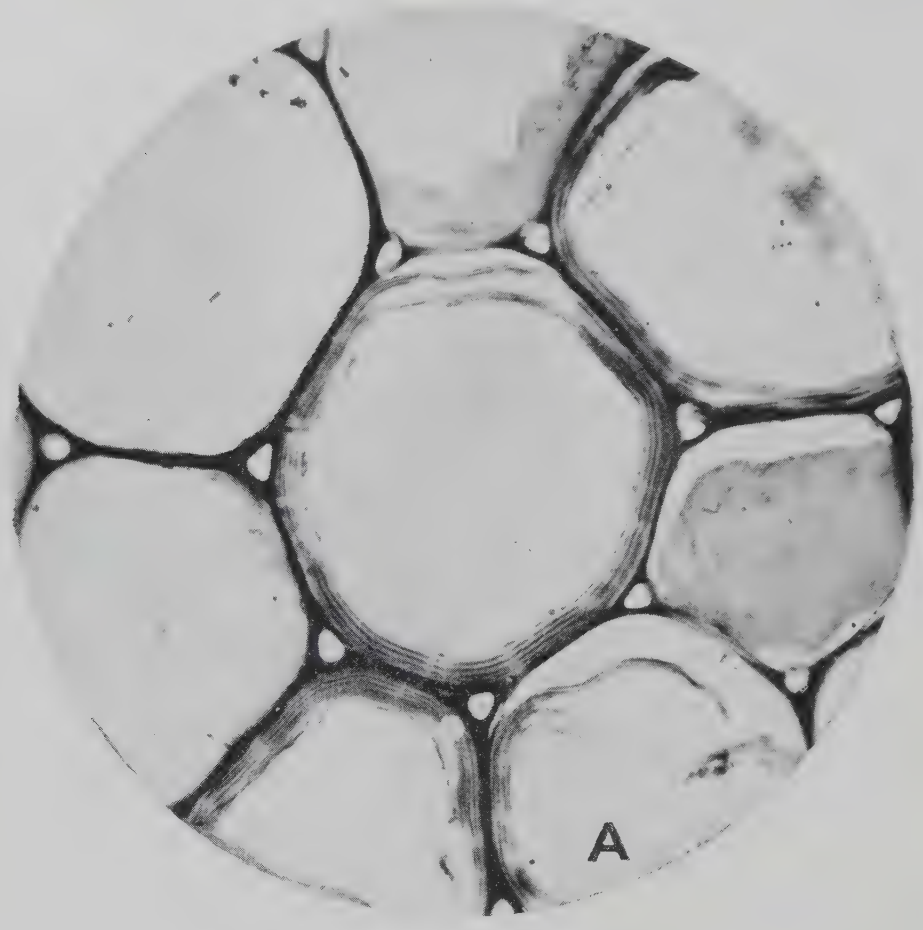
B.—Section of single upper epidermal cell of leaf and adjacent palisade cell. The epidermal cell is filled with granular tanniferous material; the palisade cell shows disorganized protoplasm, starch grains, and crystals. A small crystal is seen inside the nucleus.

C.—Section of pith cell which is transformed into a sclereid adjacent to phloem fibers. The walls of the latter are very thick and strongly lignified.


A


B


C


A

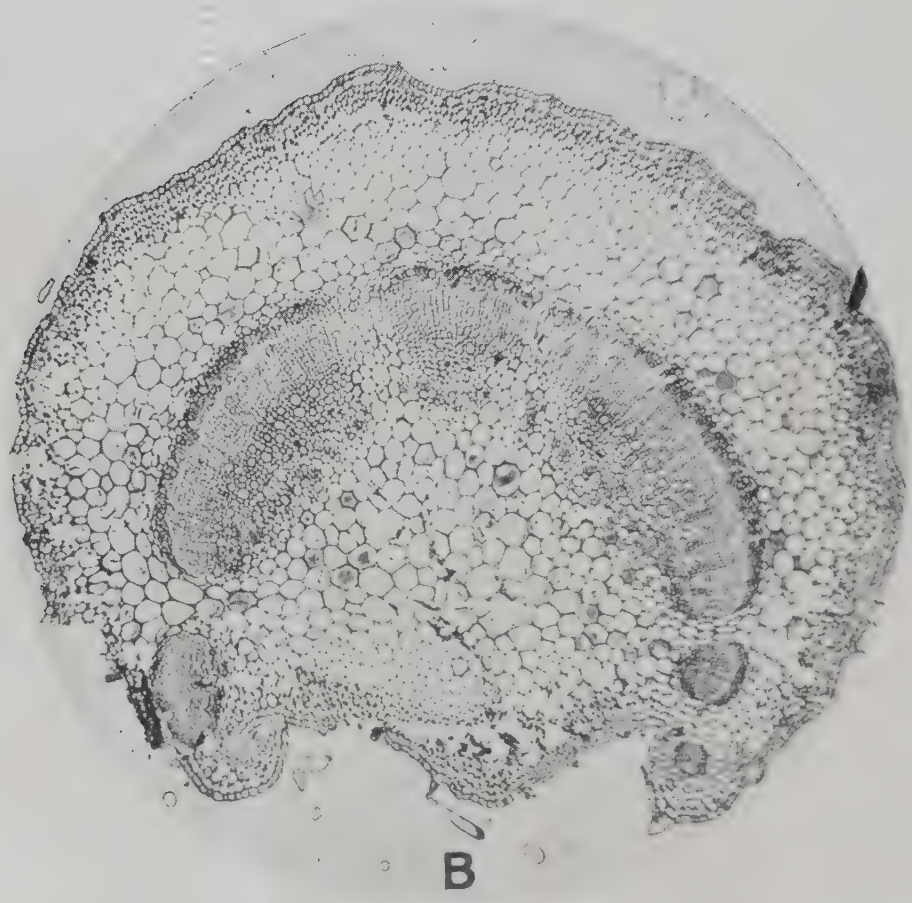

B

PLATE 58

A.—Pith cells of petiole transformed into sclereids with typically stratified walls.

B.—Vascular tissue of the petiole greatly increased by blackleg. A number of sclereids are seen in the pith.

SCLEROTINIA MINOR, N. SP., THE CAUSE OF A DECAY OF LETTUCE, CELERY, AND OTHER CROPS

By Ivan C. Jagger

Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

Smith [1] (1900) recorded the occurrence of a fungus similar to *Sclerotinia libertiana* Fuckel, which, however, produced much smaller sclerotia (Pl. 59, A) in greenhouses of Massachusetts, where it was causing a destructive rot of lettuce. Duggar [2] (1909) states that a similar fungus occurs on lettuce in the vicinity of both Boston and New York City. In 1911 the writer [3] obtained what appeared to be the same fungus from decayed lettuce grown in the vicinity of New York. It was collected in 1912 and again in 1914 at South Lima in western New York, where it seemed to be well established and was causing considerable injury to lettuce grown on muck soil. In 1914 it was also collected on lettuce in a greenhouse at Rochester, N. Y., but the fungus was not again found in that vicinity, although numerous collections of diseased lettuce were made during the next three years. In the fall of 1919 Dr. W. S. Beach of the Pennsylvania Agricultural Experiment Station advised that the fungus is frequently found on celery and lettuce in the vicinity of Philadelphia. During the winter season of 1919–20 the writer observed the fungus in destructive amounts in a single field of lettuce at Sanford, Fla. In numerous inspections of lettuce in that vicinity throughout the season the fungus was observed in no other fields, although *S. libertiana* was more or less abundant in all fields. This suggests that the fungus forming small sclerotia may have been recently introduced into that section.

The fungus causes a very rapid decay and collapse of growing lettuce plants. The disease produced is almost identical with that caused by *S. libertiana*. A soft, watery decay may begin at any point on the plant but usually on the lower leaves, which rest on the ground, or on the stem near the ground. The rot spreads very rapidly, and usually the main stem and bases of the leaves are soon involved. The result is a rather sudden collapse of the whole plant. The plant is rapidly converted to a soft, watery mass. When the decayed mass is pulled apart the spaces between and around the decayed leaves and stem are found to

[1] Smith, Ralph E. botrytis and sclerotinia: their relation to certain plant diseases and to each other. *In* Bot. Gaz., v. 29, no. 6, p. 369–407, pl. 25–27. 1900.

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be filled with white wefts of mycelium, which in a few days are replaced by numerous small black sclerotia. General observations indicate that the fungus possibly causes a rather more rapid decay and collapse of plants than is caused by *S. libertiana*. The wefts of white mycelium in decaying plants are less conspicuous, and the sclerotia are much smaller and much more numerous than in plants attacked by *S. libertiana*.

On several occasions bits of culture media covered with mycelium of the fungus have been placed on growing lettuce plants. When moist conditions have followed the inoculation, characteristic rapid decay has invariably resulted. Prof. H. H. Whetzel has found that the fungus is capable of attacking a large number of plants, data on which will be

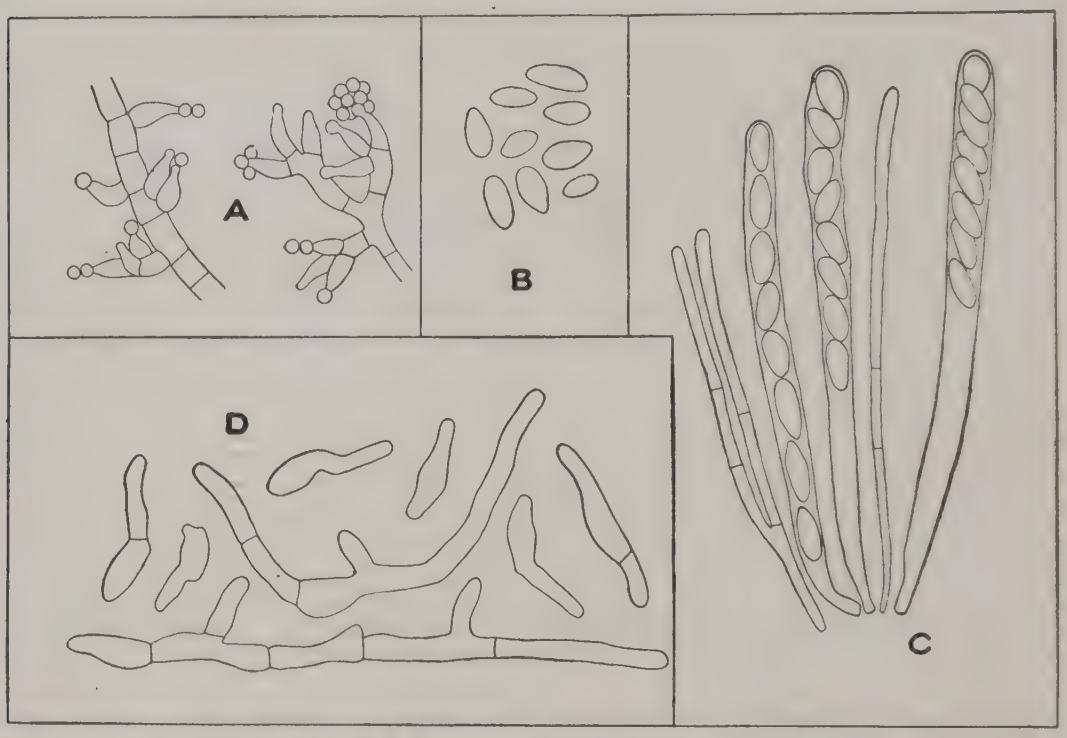


FIG. 1.—Camera lucida drawings of *S. minor*: A, microconidia and conodiophores; B, ascospores; C, germinating ascospores; D, asci and paraphyses.

published in connection with his studies of the genera Sclerotinia and Botrytis.

Strains of the fungus isolated from lettuce grown in the vicinity of New York, Rochester, and South Lima, N. Y., Philadelphia, Pa., and Sanford, Fla., have been grown in parallel cultures on various media and have in every case appeared to be identical. Apothecia produced by the three strains from New York State have shown neither macroscopic nor microscopic differences.

Apothecia (Pl. 59, B, C) have several times developed from sclerotia which had been allowed to age on unsterilized sand for from 4 to 12 months and which were then held under moist and well-lighted conditions. Studies of fresh mature apothecia were made in 1912, 1914, and 1917 (fig. 1). Measurements of spores, asci, and paraphyses in the

description are from the combined data of the three years, since the three sets of data agree very closely. Microconidia (fig. 1) have appeared in abundance on a medium consisting of a 2 per cent agar flour in distilled water. Cultures have been obtained repeatedly from single ascospores which have shown the apothecia to be the fruiting stage of the sclerotia-producing fungus.

Smith (1900) [1] in studies of this fungus was unable to obtain apothecia, although apothecia of *S. libertiana* were obtained in abundance. In hundreds of cultures the fungus developed only the characteristic small sclerotia, but in a single culture the small sclerotia at first appeared, and later the characteristic large sclerotia of *S. libertiana* appeared among the small ones. Smith believed that *S. libertiana* developed directly from the small sclerotia and, therefore, concluded that the fungus is—

a degenerate form of *S. libertiana* which has almost entirely lost the ability to reproduce by spores.

The repeated development during several years of characteristic apothecia and the fact that during 10 years numerous cultures of several strains of the fungus have shown very uniform characteristics seem sufficient grounds for considering the fungus a distinct species. As it seems to agree with no described species, the following description is given.

Sclerotinia minor, n. sp.

Apothecia one, rarely more, from a single sclerotium; disc saucer-shaped, 0.5 to 2 mm. in diameter; stalk cylindrical, slender, flexuous, attenuated downward, 5 to 12 mm. long; asci cylindrical to cylindro-clavate, 125 to 175 μ by 8 to 11 μ, average of 30 measurements 141 by 8.9 μ; spores 8, ellipsoid to ovoid, hyaline, 5 to 8.8 μ by 8.3 to 19.9 μ, average size of 200 spores 7 by 14.1 μ with over 80 per cent 6 to 8 μ by 12 to 16 μ; paraphyses filiform to cylindro-clavate, septate, rarely branched, same length as asci, 3 to 4 μ in diameter; microconidia globose, hyaline, 3 to 4.2 μ, borne apically on short obclavate conidiophores; appressoria abundant; sclerotia black, irregular, 0.5 to 2 mm. in diameter, often anastomosing to form irregular flattened bodies several millimeters in length.

Parasitic on lettuce (*Lactuca sativa* L.), celery (*Apium graveolens* L.), and other plants; distribution, Massachusetts, New York, Pennsylvania, and Florida.

SUMMARY

Sclerotinia minor, n. sp., produces a decay of lettuce and other plants similar to that produced by *S. libertiana*. It is known to occur in Massachusetts, New York, Pennsylvania, and Florida.

[1] SMITH, Ralph E. OP CIT.

PLATE 59

A.—Sclerotia on hard potato agar: center, *Sclerotinia libertiana*, either end, *S. minor*.

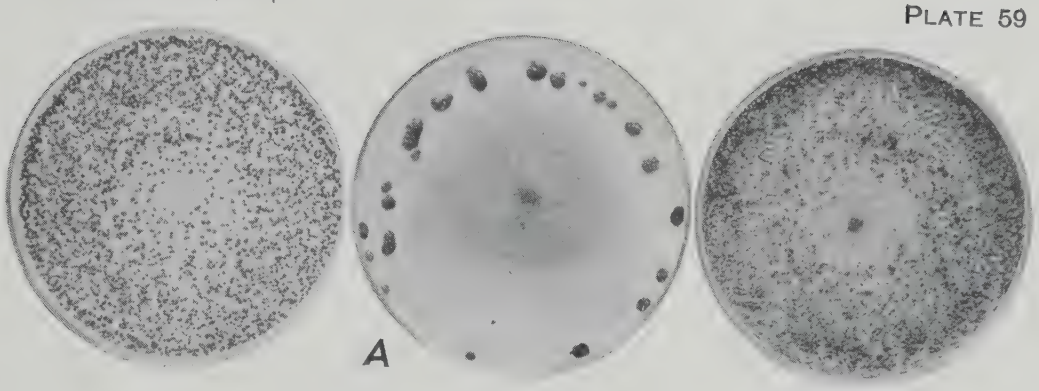
B.—Apothecia of *S. libertiana*.

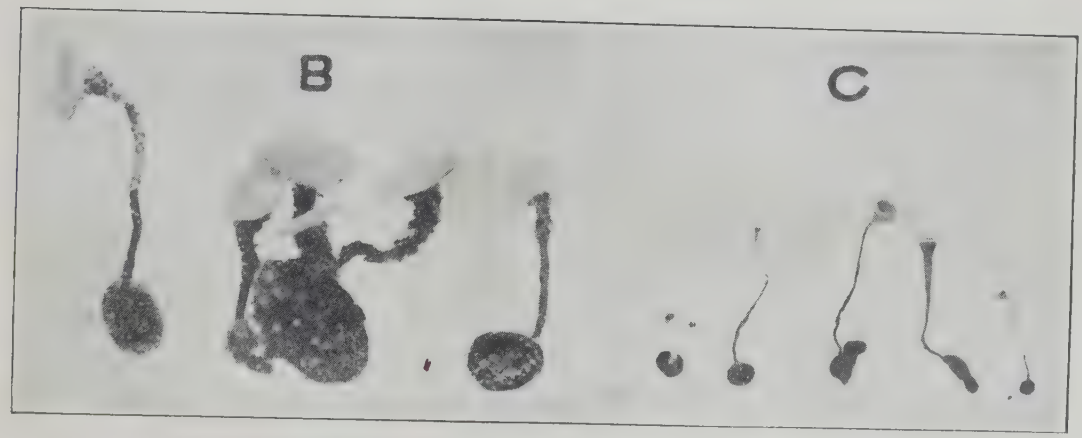
C.—Apothecia of *S. minor*.

Note relative size of apothecia in B and C.

Sclerotinia minor, n. sp.

PLATE 59





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PERMANENCE OF DIFFERENCES IN THE PLOTS OF AN EXPERIMENTAL FIELD

By J. ARTHUR HARRIS, *Investigator, Station for Experimental Evolution, Cold Spring Harbor, N. Y., and Collaborator, Office of Western Irrigation Agriculture* and C. S. SCOFIELD, *Agriculturist in Charge, Office of Western Irrigation Agriculture Bureau of Plant Industry, United States Department of Agriculture*

I.—INTRODUCTION

Agronomists have long recognized the fact that the plots of an experimental field may differ considerably among themselves. This variability is the source of the greatest difficulty in the interpretation of comparative cultures. A recent analysis (*3*)[1] of the available data by adequate biometric formulae (*1*) has shown that heterogeneity is a practically universal characteristic of experimental fields and that it must be considered in the interpretation of the results of all plot tests.

With the demonstration of this characteristic of experimental areas the questions naturally arise: Are the differences between plots transient or are they relatively permanent from year to year? Do these differences tend to increase or to decrease with cultivation?

Presumably the differences which obtain in the soil of an experimental field are in part permanent and in part transient. Lyon (*5*) suggested that tillage and other factors will change the plots so that the results will not be comparable from year to year. Unfortunately he does not present data to show to what extent this may be true. He gives a series of yields for successive years on the same plots, which measured 33 by 66 feet or $^1/_{20}$ of an acre in area, at the Nebraska Agricultural Experiment Station and shows that the rank of the yield of these plots differs greatly from year to year. Thus he concludes that if they differ among themselves in their capacity for crop production this difference is very little constant from year to year.

Smith (*6*) took advantage of the breaking up of a piece of land which had lain 16 years in pasture to investigate the effect of cultivation on the uniformity of a series of plots. Any influence of 1 or 2 years preceding cultures on the variation or correlation of yields should, he assumed, be apparent in the statistical constants deduced from these

[1] Reference is made by number (italic) to "Literature cited," p. 356.

data. He gives a table which indicates that there is such a change. He says:

> It is noticeable that the variability as measured by the standard deviation becomes less in each succeeding year. This suggests the question as to whether continued cropping might not tend to induce uniformity. The records of a few of these plots which were continued in corn for three years longer do not support such a conclusion.

It must be noted that in Smith's experiments seasonal conditions varied greatly from year to year. Thus 1895, which was exceedingly dry and also cool in the early part of the season, was highly unfavorable. The two following years were unusually favorable for corn. As a result the yields were, respectively, 31.6, 91.6, and 71.4 bushels per acre in the three years.

Lehmann in his work at the experimental farm near Bangalore attempted to use the experience of previous years in the standardization of experimental plots. His data will be considered in some detail below.

II.—METHODS AND RESULTS

The permanency of the differentiation of plots in their capacity for crop production may be measured in terms of correlation. If the plots of a field differ among themselves in a more or less permanent way there will, with reasonably uniform climatic conditions, be a correlation between the yields of the plots of a series in two or more successive years—in short, an intera nnual correlation (*2*).

The problem of the correlations between the yields of identical plots in different years is one of very great interest. If this correlation be high it should be possible to standardize a field of plots by one or more sowings to the same variety. A chief difficulty in the standardization of the field by the carrying out of a preliminary test in which the productive capacities of the plots are determined once and for all lies in the fact that the factors which determine yield are in part edaphic—that is, pertaining to soil conditions—and in part meteorological. For example, in a very dry year sections of a field which are lower may produce the heaviest crops because adequate moisture is longer retained in these places. In a wet year the case may be just the reverse, for the crops in the lower-lying portions may be too wet for the best plant growth. Thus, it is quite possible that in cases in which there is a profound influence of environmental factors there may be a negative correlation between the yield of the same plots in different years.

It is conceivable, therefore, that the interannual correlation for yield per plot may range from negative to positive values, zero correlation being found in cases in which edaphic and meteorological factors exactly counterbalance each other in their influence upon the yield of the plots of a heterogeneous field.

A.—PUBLISHED DATA

Unfortunately few data are available for analysis from the literature. Lehmann has given (*4, p. 6*) yields of paddy on the 17 plots of ranges B and C, respectively, of the wet tract of the Experimental Farm at Hebbel. Grouping the yields for the two ranges, we find for the correlations between the yields of the same plots in the two years 1905 and 1906:

Range B, $r = 0.834 \pm 0.050$, $r/Er = 16.7$.
Range C, $r = 0.799 \pm 0.059$, $r/Er = 13.5$.

Stockberger (7) gives data for the extremes of a series of hill yields for hops. The interannual correlations deduced from these data have been shown (*2*) to be as follows:

Years.	Lowest hills.	Highest hills.
1909 and 1910	0.29±0.17	0.59±0.13
1910 and 1911	.55± .13	.52± .14
1909 and 1911	.43± .15	.30± .18

Stockberger has also given (*8*) the yields for 30 rows, each 210 feet in length, from hop fields of several hundreds of acres in the Sacramento Valley of California:

> The plants in these rows averaged well in number and uniformity of growth with the plants on several hundreds of acres of hops in the midst of which the experimental area was located.

Data are available for the years 1909 to 1914. Calculating the correlation between the yields in the different years, we have the results set forth in Table I. It appears that with one single exception the constants are positive throughout. In general they are significant in comparison with their probable errors, indicating a superiority in a subsequent year if a superiority is shown in a given year.

The constants in the table are arranged in a way to show the change in the coefficient of correlation as the years become more widely separated in time. Thus, in the case of the correlation for the 1909 yields, the constant for "first and second" is that showing the relationship between the 1909 and 1910 yields, while "first and third" indicates the constant measuring the relationship between the yields of 1909 and 1911. Similarly, in the series of coefficients for 1910 "first and second" designates the correlation between 1910 and 1911, etc.

For the series beginning with 1909 we note a marked decrease in the magnitude of the constants as the yields correlated become more widely separated in time. The same is true for the series beginning with 1910. The other series are more irregular.

TABLE I.—*Interannual correlations for yield of hops*

Beginning of series.	First and second years.	First and third years.	First and fourth years.	First and fifth years.	First and sixth years.
1909	+0.768±0.051	+0.622±0.075	+0.380±0.105	+0.259±0.115	+0.061±0.123
1910	+ .577± .082	+ .447± .099	+ .451± .098	+ .274± .114	.....
1911	+ .062± .123	+ .313± .111	− .126± .121	.....	.....
1912	+ .311± .111	+ .705± .062	.....	.....	.....
1913	+ .597± .079	.....	.....	.....	.....

The most reasonable explanation of the higher correlation of more closely associated years is that both field conditions and the productiveness of the individual vines change more or less as time goes on. The result of such changes would be a lower correlation between the yields of periods more widely separated in time.

The data for the dry-land experiments in Mysore State have been discussed elsewhere (*3*) in relation to the problem of field heterogeneity. It was shown there that in two dry years the field showed marked hetereogeneity, but that in one unusually wet season there was marked abnormality of yield with little correlation between the yields of adjacent plots.

It seems of unusual interest, therefore, to determine to what extent the differences between these plots are permanent from year to year. Correlating between the yields of ragi, we find the following correlation coefficients for the whole series of 105 plots for which data are available.

Years.	Grain.	Straw.	Total.
1905 and 1906	0.591±0.043	0.777±0.026	0.757±0.028
1905 and 1907	.693± .034	.855± .018	.852± .018
1906 and 1907	.450± .052	.678± .036	.610± .041

The correlations are of very substantial order, and without exception they are clearly significant in comparison with their probable errors. They show that the differences in the plots are to a high degree persistent during the three years of this experiment.

For grain, straw, and total yield the correlations between the yield for 1905 and 1907 are higher than those for 1905 and 1906 or for 1906 and 1907. If there were a progressive change in the field one might have expected that the correlations would be higher between consecutive years. Apparently the influence of the abnormal conditions of 1906 has been to lower the correlations for this year.

The results show that the capacity for production is to a high degree persistent from year to year, notwithstanding great diversity in meteorological conditions.

A series of records of unusual interest is provided by Smith (*6*) for yields of corn in three successive years, 1895, 1896, 1897. It has been

shown elsewhere (*3*) that this field, which had lain in grass for many years, is highly heterogeneous, showing correlations between adjacent plots of $r=0.61$ to $r=0.83$.

The conditions for corn production differed very greatly in the three years. Thus the constants for yield were:

Year.	Mean.	Standard deviation.	Coefficient of variation.
1895	31.7	7.91	25.0
1896	91.6	10.64	11.6
1897	71.4	6.27	8.8

Yield is over twice as heavy in the second and third years as in the first. The variability in yield as measured by the coefficient of variation is far lower in the second and third years than in the first.

Computing the correlations between the yields for the three years, we have the following results:

For 1895 and 1896, $r=-0.354\pm0.054$, $r/E_r=6.6$.

For 1895 and 1897, $r=-0.221\pm0.059$, $r/E_r=3.8$.

For 1896 and 1897, $r=+0.818\pm0.020$, $r/E_r=40.1$.

There is a negative correlation for 1895 and 1896 and for 1895 and 1897 but a high positive correlation for 1896 and 1897. Thus the plots which were better in the highly unfavorable year 1895 were poorer in the two favorable years 1896 and 1897. Plots which were better in the favorable year 1896 were also better in the favorable year 1897.

B.—THE HUNTLEY UNIFORM CROPPING EXPERIMENT

The most extensive series of records available is that for a uniform cropping experiment conducted for the past several years at the Field Station of the Office of Western Irrigation Agriculture, at Huntley, Mont.

The Huntley field lies in the Yellowstone Valley on land having a very slight and uniform slope to the north. The detailed history of the field prior to 1910 is not known definitely. It was probably first broken from the native prairie sod in the spring of 1908. In 1909 it was planted to sugar beets, but the crop was destroyed in the late summer. It came under experimental control in 1910, when the major portion of it was sown to oats and yielded a crop of 66 bushels per acre. In that season a small tract in the northeast corner of the field was used as a machinery park or stack yard and was not put into crop. This tract occupied about two-thirds of the length of the first five plots in series II. It is possible that this difference of treatment in 1910 may have been reflected in the crop yields of 1911, but it seems hardly probable that any material effects could have persisted longer.

In the spring of 1911 this field was laid out into 46 plots, each measuring 23½ by 317 feet and containing 0.17 acre, arranged in two parallel series of 23 plots each. The two series of plots were separated merely by a temporary irrigation ditch. In 1911 it was planted to sugar beets, and in the spring of 1912 it was seeded to alfalfa, and one cutting was harvested that year. This stand remained on the ground during 1913 and 1914, when the entire field was fall-plowed. In 1913 three cuttings were made, but the third cutting was lost in a heavy wind which scattered and mixed the crop before weighings from the various plots could be made. The first cutting, designated as alfalfa I, was made on plots one-half the original size. The second cutting was harvested from plots one-quarter the original size. The first and second cuttings in 1914 were weighed for plots one-quarter the original size—that is, 0.0425-acre plots—while the third cutting was recorded for plots one-third the original size. These furnish the data for alfalfa I, II, and III for 1914. Total yields for the first and second cuttings in 1913 and 1914 and for the first, second, and third cuttings in 1914 are also considered.

In 1915 and 1916 ear corn was grown. In 1917[1] the fields were planted to oats, and records were made of grain, straw, and total yield. In 1918 silage corn was grown. In 1919 the land produced a crop of barley.

It has been the practice each season to treat the whole field as a unit until harvest time, when the plot boundaries are established in order to measure the crop yields. In other words, all cultural operations, including irrigation, are carried out on a field scale and uniformly throughout the field. No manuring has so far been attempted. An effort has been made to avoid any artificial causes of heterogeneity.

The crop yields each year have been satisfactory—that is, they have not been abnormal—as is shown in Table II, where the mean yields per plot and per acre are set down. Fortunately, this experiment has also escaped injury from insect pests, plant diseases, and storms, which so often interfere with the success of long-term field experimentation.

[1] Because of other activities the plots could not be harvested in halves and quarters in 1917–1919.

TABLE II.—*Mean yields of the Huntley uniform cropping experiment*

Crop.	Number of pounds per plot.	Number of tons or bushels per acre.
1911, sugar beets	4,179.00	12.29
1912, total alfalfa	356.54	1.04
1913, alfalfa I	541.41	1.59
1913, alfalfa II	483.26	1.42
1913, alfalfa I and II	1,024.67	3.01
1914, alfalfa I	489.13	1.44
1914, alfalfa II	499.34	1.47
1914, alfalfa I and II	988.47	2.91
1914, alfalfa III	471.95	1.38
1914, alfalfa I to III	1,460.43	4.29
1915, ear corn	522.58	52.7
1916, ear corn	396.15	41.6
1917, oat grain	555.80	102.1
1917, oat straw	521.54	1.53
1917, total yield	1,077.34	3.16
1918, silage corn	3,175.43	9.34
1919, barley grain	358.19	43.8
1919, barley straw	230.50	.67
1919, total yield	588.69	1.73

The data furnished by this series of records are of particular value, since (*a*) they are based on irrigated plots and (*b*) it is possible to compare the correlations between the same crop and different crops in the different years.

The correlations between the yields of the various crops in the different years may be considered in three series.

(1) The first comprises the yields for the whole plots. In this series we determine the correlation between the crop produced on the 46 plots in one year and that produced on the same 46 plots in another year.

(2) In the study of certain crops the plots were divided into two subplots, and we may determine the relationship between yield of individual subplots in different years. Then the number of observations is twice what it was in the preceding correlation, that is, $N = 92$ instead of 46.

(3) Finally, in a more limited series of cases the 46 original plots were harvested in 4 subplots each, thus increasing the number of units which may be entered in the correlation tables to 184.

The data for determining the correlations between yields of various crops for the 46 whole plots are given in Table III. The data for half plots and quarter plots may be obtained from the diagrams in an earlier paper by Harris (*3*) on the practical universality of field heterogeneity as a factor affecting plot yields. The correlation coefficients and their probable errors for whole plots are shown in Table IV.

TABLE III.—*Yield of plots of field B at the Huntley (Mont.) Field Station* [a]

Plot No.	1911, sugar beets.	1912, total alfalfa.	1913, alfalfa I.	1913, alfalfa II.	1913, alfalfa I and II.	1914, alfalfa I.	1914, alfalfa II.	1914, alfalfa I and II.	1914, alfalfa III.	1914, alfalfa I to III.
II, 1	12.78	260	595	600	1,195	585	550	1,135	580	1,715
2	12.70	395	530	560	1,090	610	605	1,215	605	1,820
3	10.04	397	640	630	1,270	605	690	1,295	595	1,890
4	10.35	435	640	650	1,290	640	660	1,300	610	1,910
5	9.33	442	625	540	1,165	590	700	1,290	595	1,885
6	9.40	419	625	595	1,220	645	735	1,380	510	1,890
7	11.53	438	640	575	1,215	625	775	1,400	500	1,900
8	12.40	410	555	570	1,125	555	725	1,280	500	1,780
9	10.28	418	570	470	1,040	590	615	1,205	475	1,680
10	11.81	393	540	470	1,010	545	505	1,050	450	1,500
11	13.99	405	585	435	1,020	580	430	1,010	400	1,410
12	12.28	435	530	450	980	555	425	980	445	1,425
13	11.91	385	565	485	1,050	465	445	910	455	1,365
14	11.42	395	555	510	1,065	540	480	1,020	485	1,505
15	12.28	405	655	565	1,220	535	515	1,050	540	1,590
16	13.76	305	650	475	1,125	545	440	985	475	1,460
17	11.73	312	590	435	1,025	540	435	975	460	1,435
18	12.49	290	635	425	1,060	540	525	1,065	455	1,520
19	15.55	315	635	455	1,090	545	490	1,035	465	1,500
20	11.93	310	605	440	1,045	540	505	1,045	475	1,520
21	13.52	330	625	455	1,080	580	535	1,115	510	1,625
22	14.36	325	625	500	1,125	610	525	1,135	520	1,655
23	16.81	310	590	425	1,015	490	445	935	380	1,315
III, 1	13.93	405	535	425	960	420	470	890	645	1,535
2	13.04	350	470	430	900	430	395	825	520	1,345
3	10.55	400	510	405	915	395	435	830	495	1,325
4	11.63	435	475	425	900	440	450	890	440	1,330
5	10.56	350	460	445	905	435	420	855	455	1,310
6	10.00	365	510	510	1,020	430	375	805	415	1,220
7	10.54	390	500	440	940	410	400	810	445	1,255
8	10.00	325	455	425	880	415	380	795	415	1,210
9	8.85	360	490	375	865	425	410	835	385	1,220
10	10.48	360	440	415	855	365	390	755	360	1,115
11	12.61	335	485	390	875	360	420	780	385	1,165
12	11.22	350	470	400	870	360	430	790	370	1,160
13	12.08	370	500	450	950	390	505	895	435	1,330
14	11.91	255	470	485	955	370	495	865	425	1,290
15	12.65	370	485	455	940	380	470	850	455	1,305
16	11.71	325	460	440	900	360	455	815	410	1,225
17	12.19	280	460	445	905	395	425	820	430	1,250
18	12.62	280	430	500	930	395	425	820	385	1,205
19	13.45	320	480	515	995	450	515	965	475	1,440
20	15.60	275	520	565	1,085	435	480	915	445	1,360
21	16.25	290	460	510	970	435	480	915	465	1,380
22	14.70	345	530	535	1,065	475	515	990	495	1,485
23	16.52	337	505	530	1,035	475	475	950	475	1,425

[a] All yields are given in pounds per plot with the exception of that for sugar beets, which is given in tons per acre.

TABLE III.—*Yield of plots of field B at the Huntley (Mont.) Field Station* [a]—Con.

Plot No.	1915, ear corn.	1916, ear corn.	1917, oat grain.	1917, oat straw.	1917, total yield.	1918, silage corn.	1919, barley grain.	1919, barley straw.	1919, total yield.
II, 1	556	513	580	574	1,154	3,655	392	288	680
2	598	514	593	631	1,224	3,285	349	251	600
3	526	481	606	588	1,194	3,290	377	253	630
4	558	495	598	414	1,012	3,390	352	218	570
5	509	487	614	590	1,204	3,570	414	246	660
6	521	450	596	584	1,180	3,240	426	264	690
7	499	489	572	458	1,030	3,005	463	262	725
8	502	441	574	524	1,098	3,010	424	276	700
9	515	434	553	495	1,048	3,060	425	265	690
10	513	415	614	606	1,220	2,885	422	298	720
11	524	399	574	578	1,152	2,955	386	224	610
12	507	379	548	510	1,058	3,055	365	240	605
13	528	376	537	523	1,060	3,125	350	220	570
14	507	372	540	522	1,062	3,210	368	222	590
15	511	398	518	616	1,134	3,155	344	191	535
16	524	409	564	570	1,134	2,870	351	204	555
17	520	389	499	481	980	2,950	333	127	460
18	479	408	538	518	1,056	3,235	309	241	550
19	455	404	637	605	1,242	3,330	313	177	490
20	489	383	579	497	1,076	3,150	304	221	525
21	519	455	567	513	1,080	3,180	316	229	545
22	573	413	553	477	1,030	3,075	306	199	505
23	578	414	509	391	900	3,375	288	257	545
III, 1	545	404	563	547	1,110	3,685	332	238	570
2	552	376	560	522	1,082	3,365	362	218	580
3	504	337	511	511	1,022	3,315	375	260	635
4	547	318	523	497	1,020	3,170	342	183	525
5	544	338	532	516	1,048	3,240	416	284	700
6	533	312	536	552	1,088	3,290	460	250	710
7	505	311	538	544	1,082	2,855	410	330	740
8	519	345	552	556	1,108	2,905	400	260	660
9	513	353	515	535	1,050	2,965	400	260	660
10	509	337	521	545	1,066	2,760	386	274	660
11	493	322	473	479	952	2,640	403	262	665
12	496	357	520	462	982	2,850	305	255	560
13	503	343	645	377	1,022	2,880	296	199	495
14	496	333	525	469	994	3,190	290	130	420
15	518	360	557	485	1,042	3,100	301	174	475
16	499	372	578	504	1,082	2,975	335	185	520
17	483	353	549	515	1,064	2,995	317	188	505
18	469	367	563	517	1,080	3,315	320	190	580
19	477	410	562	512	1,074	3,540	293	187	400
20	490	407	561	481	1,042	3,280	323	177	590
21	551	426	486	456	942	3,370	331	259	580
22	628	423	573	571	1,144	3,625	362	218	510
23	654	401	561	573	1,134	3,705	341	249	590

[a] All yields are given in pounds per plot with the exception of that for sugar beets, which is given in tons per acre.

From the series of correlations as a whole it appears that of the 152 coefficients showing the relationship between crop yields in different years, 133 are positive while only 19 are negative in sign. If the differences in capacity for crop production demonstrated in different years were due to purely transient causes, one would expect to find an approximately equal number of positive and negative correlations with the general average value sensibly zero. Instead we find the proportion of 133 to 19. This is a deviation from the ratio 76 to 76, which one might expect on the assumption that there is no correlation between the yields of plots in a series of years, of

$$57 \pm 0.6745\sqrt{152 \times 5 \times 0.5} = 57 \pm 4.16.$$

The deviation from equality is 13.7 times as large as its probable error and is unquestionably significant.

If we consider that coefficients which are 2.5 times or more as large as their probable errors represent statistically significant interrelationships, we find that of the 82 relationships which may be regarded as falling in this class 78 are positive whereas only 4 are negative in sign.

Averaging the values of the coefficients considered in Table IV, we note that the average for the 133 positive values is +0.3346, whereas that for the 19 negative values is −0.1475. Taking the constants altogether, the average value is +0.2743.

There is, therefore, an overwhelming body of evidence to show that plots, even of the small size and the apparent uniformity of those of the Huntley Station, which yield higher in one year will yield higher persistently throughout a series of years.

It is now desirable to determine whether the same relationships hold when these plots are divided into smaller subplots. It is possible to subdivide a number of the plots into 2 subplots, each one-half the original size. Correlations may be determined for the 92 yields of these half plots in the same manner as for the total yields on the 46 original plots. The results appear in Table V.

The constants are positive throughout. In general, they are statistically significant in comparison with their probable errors. As a matter of fact, only 2 of the 22 constants are less than twice as large as their probable errors. Thus, they indicate a real biological relationship between the productions of the half plots in different years. Those which give a higher yield in one year give a higher yield in another year.

For a smaller number of the crops it is possible to divide the original plots into quarter plots, thus securing 184 subplots to be used as a basis of calculation. The coefficients of correlation between the yields in the several years are shown in Table VI.

TABLE V.—*Interannual correlations for yield of 92 half plots in the Huntley uniform cropping experiment*

	1913, alfalfa I.	1913, alfalfa II.	1913, alfalfa I and II.	1914, alfalfa I.	1914, alfalfa II.	1914, alfalfa I and II.	1915, ear corn.	1916, ear corn.
1913, alfalfa I				+0.762±0.030 +25.8	+0.543±0.050 +10.9	+0.715±0.034 +20.8	+0.125±0.069 +1.80	+0.686±0.037 +18.4
1913, alfalfa II				+.438±.057 +7.71	+.601±.045 +13.4	+.580±.047 +12.4	+.229±.067 +3.43	+.618±.043 +14.2
1913, alfalfa I and II				+.707±.035 +20.1	+.673±.038 +17.5	+.763±.029 +25.9	+.208±.067 +3.09	+.768±.029 +26.6
1914, alfalfa I	+0.762±0.030 +25.8	+0.438±0.057 +7.71	+0.707±0.035 +20.1				+.228±.067 +3.42	+.744±.031 +23.7
1914, alfalfa II	+.543±.050 +10.9	+.601±.045 +13.4	+.673±.038 +17.5				+.112±.069 +1.61	+.739±.032 +23.2
1914, alfalfa I and II	+.715±.034 +20.8	+.580±.047 +12.4	+.763±.029 +25.9				+.185±.068 +2.72	+.820±.023 +35.7
1915, ear corn	+.125±.069 +1.80	+.229±.067 +3.43	+.208±.067 +3.09	+.228±.067 +3.42	+.112±.069 +1.61	+.185±.068 +2.72		+.329±.063 +5.25
1916, ear corn	+.686±.037 +18.4	+.618±.043 +14.2	+.768±.029 +26.6	+.744±.031 +23.7	+.739±.032 +23.2	+.820±.023 +35.7	+.329±.063 +5.25	

TABLE VI.—*Interannual correlations for yield of 184 quarter plots in the Huntley uniform cropping experiment*

	1913, alfalfa II.	1914, alfalfa I.	1914, alfalfa II.	1914, alfalfa I and II.	1915, ear corn.	1916, ear corn.
1913, alfalfa II		+0.373±0.043 +8.71	+0.373±0.043 +8.71	+0.446±0.040 +11.2	+0.193±0.048 +4.04	+0.512±0.037 +14.0
1914, alfalfa I	+0.373±0.043 +8.71				+.174±.048 +3.61	+.642±.029 +22.0
1914, alfalfa II	+.373±.043 +8.71				+.109±.049 +2.22	+.629±.030 +21.0
1914, alfalfa I and II	+.446±.040 +11.2				+.158±.048 +3.27	+.719±.024 +30.0
1915, ear corn	+.193±.048 +4.04	+.174±.048 +3.61	+.109±.049 +2.22	+.158±.048 +3.27		+.329±.044 +7.42
1916, ear corn	+.512±.037 +14.0	+.642±.029 +22.0	+.629±.030 +21.0	+.719±.024 +30.0	+.329±.044 +7.42	

Unfortunately the number of crops which can be included in Table VI is rather small. The coefficients are positive in sign throughout, and in all cases they are statistically significant in comparison with their probable errors. The individual constants will receive attention in the following discussion.

The fact that the yields are correlated in the different years for whole plots of 0.17 acre, for half plots of 0.085 acre, and for quarter plots of only 0.0425 acre emphasizes the permanence of the substratum differences. We now have to compare the correlations secured for these three divisions. The difference in the actual magitudes of the correlations appear in Table VII. The three entries, when all comparisons are possible, show: (1) the difference between the correlation for whole plots and half plots, (2) the difference between the correlation for whole plots and quarter plots, and (3) the difference between the correlation for half plots and quarter plots.

The signs are positive when the correlations are larger for the larger areas.

The comparisons show that in general the correlations decrease in magnitude as the areas upon which they are based are subdivided. Thus 16 of the 22 comparisons of the correlations deduced from whole plots and from half plots (first entry) show a lower correlation in the half plots as compared with 6 which show higher correlations in the half plots.

TABLE VII.—*Differences in interannual correlations for whole plots, half plots, and quarter plots*

	1913, alfalfa I.	1913, alfalfa II.	1913, alfalfa I and II.	1914, alfalfa I.	1914, alfalfa II.	1914, alfalfa I and II.	1915, ear corn.	1916, ear corn.
1913, alfalfa I		.....	.....	−0.0863	−0.0514	−0.0558	+0.0910	+0.0387
		.....	.....	.....	.....	.....	.....	.....
		.....	.....	.....	.....	.....	.....	.....
1913, alfalfa II	.....		.....	− .1622	− .1335	− .1462	− .0267	− .1021
	.....		.....	− .2276	− .3615	− .2794	− .0623	− .2081
	.....		.....	− .0653	− .2280	− .1332	− .0355	− .1059
1913, alfalfa I and II	.....	.....		− .1442	− .1044	− .1152	+ .0403	− .0330
	.....	.....		.....	.....	.....	.....	.....
	.....	.....		.....	.....	.....	.....	.....
1914, alfalfa I	−0.0863	−0.1622	−0.1442		.....	.....	+ .0388	− .0560
	.....	− .2276	.....		.....	.....	− .0150	− .1578
	.....	− .0653	.....		.....	.....	− .0539	− .1018
1914, alfalfa II	−0.0514	− .1335	− .1044	.....		.....	+ .0705	− .0493
	.....	− .3615	.....	.....		.....	+ .0676	− .1599
	.....	− .2280	.....	.....		.....	− .0029	− .1105
1914, alfalfa I and II	−0.0558	− .1462	− .1152	.....	.....		+ .0649	− .0377
	.....	− .2794	.....	.....	.....		+ .0385	− .1388
	.....	− .1332	.....	.....	.....		− .0264	− .1011
1915, ear corn	+0.0910	− .0267	+ .0403	+ .0388	+ .0705	+ .0649		− .0492
	.....	− .0623	.....	− .0150	+ .0676	+ .0385		+ .0487
	.....	− .0355	.....	− .0539	− .0029	− .0264		− .0004
1916, ear corn	+0.0387	− .1021	− .0330	− .0560	− .0493	− .0377	− .0492	
	.....	− .2081	.....	− .1578	− .1599	− .1388	+ .0487	
	.....	− .1059	.....	− .1018	− .1105	− .1011	− .0004	

Of the 12 comparisons possible between the interannual correlations deduced from whole plots and from quarter plots (second entry), 9 show lower correlations for quarter plots as compared with 3 which show higher correlations for the quarter plots. Finally, all 12 of the correlations deduced from quarter plots are lower than the correlations deduced from half plots.

It appears, therefore, that 0.085 and 0.0425 acre are rather too small to give the highest values of the interannual correlations. On areas of this size other factors than the peculiarities of the plots themselves have too large an influence upon variation of yield to allow the individuality of the plots to express itself fully in its influence upon the yields of successive years.

In support of the conclusion that the lower value of the correlations for half and quarter plots is due to the greater variability of the yields of these plots we note that the coefficients of variation for subplots are without exception larger than those for the plots of the original size. The coefficients of variation are as follows for the years in which the plots were subdivided.

Crop.	Whole plots.	Half plots.	Quarter plots.
1913, alfalfa I	12.52	14.93	.....
1913, alfalfa II	13.60	16.59	21.87
1913, alfalfa I and II	11.11	13.34	.....
1914, alfalfa I	17.94	20.04	23.68
1914, alfalfa II	19.81	21.77	25.87
1914, alfalfa I and II	17.47	18.90	21.88
1915, ear corn	7.29	8.43	9.23
1916, ear corn	13.43	15.88	17.68

It is now desirable to examine the results for the individual crops. In doing this it may be noted that there are two factors to be taken into account. First, there is the possibility of an inherent difference in the plots which is persistent from year to year and is quite independent of the crop grown. Second, it is conceivable that the crop itself may exert an influence upon the soil such that the yields of subsequent crops will be influenced by variations in its growth which are measured in terms of yield.

The first of these factors would influence all correlations between plots—those between the yields of given years and the yields of both preceding and subsequent seasons. The second would influence only correlations with subsequent years.

In a series of only 46 plots it will probably be impossible to distinguish between the influences of these two factors.

We note that the higher yields of beets are followed by lower yields of alfalfa in 1912, but that there is practically no relationship between the yields of sugar beets in 1911 and the yield of other crops on the same

plots from 1913 to 1918. Possible exceptions are ear corn in 1915 and silage corn in 1918, for which the correlations are positive and perhaps statistically significant in comparison with their probable errors. The correlations for yields of sugar beets in 1911 and yields of barley in 1919 are negative in sign and apparently statistically significant in comparison with their probable errors. We have no explanation to offer concerning this apparent relationship. The average value, with regard to sign, of the correlations between the yield of sugar beets and other crops is -0.077.

The correlations between the 9 different cuttings of alfalfa made during 1912 to 1914 and all other yields are generally positive and statistically significant in comparison with their probable errors. The only exceptions are the negative correlation with sugar beets in 1911 which have already been noted and the slight and statistically insignificant correlation for the 1912 yield of alfalfa and the yield of silage corn in 1918.

Since it is quite reasonable to assume that in a crop harvested more than once a year thickness of stand and variation in the size of the individual plants will have a large influence on the yields of the different plots in the same year, the correlations between the different cuttings of the same year as well as those between single cuttings and totals of two or more cuttings in the same year have been omitted from the tables. The correlations between different cuttings in the same year are given in Table VIII.

TABLE VIII.—*Comparison of correlations between different cuttings of alfalfa in the same year*

Cuttings of alfalfa.	Whole plots.	Half plots.	Quarter plots.
1913, first and second cuttings..	+0.454±0.079	+0.442±0.057	.....
1914, first and second cuttings..	+ .711± .049	+ .633± .042	+0.558±0.034
1914, first and third cuttings....	+ .595± .064	.....	.....
1914 (first plus second) and third cuttings.....	+ .653± .057	.....	.....

We shall now consider the correlations between the yields of alfalfa and between the yields of alfalfa and of other crops on the same plots in different years. The individual constants may be studied in the fundamental table (Table IV). The averages are given in Table IX. This shows that the correlations between different cuttings of alfalfa are on the average larger throughout than those between the yield of alfalfa and the yields of other crops on the same plots.

TABLE IX.—*Comparison of correlations between different yields of alfalfa with correlations between yields of alfalfa and yields of other crops*

Cuttings of alfalfa.	With other cuttings of alfalfa.	With yields of other crops.	Difference.
1912, single cutting	+0.331	+0.171	+0.160
1913, first cutting	+ .611	+ .187	+ .424
1913, second cutting	+ .604	+ .282	+ .322
1913, first and second cuttings	+ .720	+ .274	+ .446
1914, first cutting	+ .666	+ .295	+ .371
1914, second cutting	+ .629	+ .244	+ .385
1914, first and second cuttings	+ .699	+ .290	+ .409
1914, third cutting	+ .524	+ .303	+ .221
1914, first, second, and third cuttings	+ .706	+ .316	+ .390

It is clear, therefore, that either stand or specific adaptation of the individual plots to alfalfa influences to an unusual degree the closeness of correlation between the yields of the plots of alfalfa in the different years.

In the first crop of ear corn (1915) we find higher yields of ear corn in 1916, a negligible difference in the yield of oat grain and straw and total yield in 1917, higher yield of silage corn in 1918, and slightly but not significantly higher yield of barley grain, straw, and total yield in 1919 following higher yield of corn in 1915.

Turning to the constants for ear corn in 1916, we note that higher yields of grain in this year are followed by higher yields of oat straw and grain in 1917 and of silage corn in 1918, and by slightly higher yields of barley grain and straw in 1919.

The average value of the correlation between the yield of ear corn in 1915 and the yield of other crops during the eight years is +0.167, whereas that for ear corn in 1916 and other crops is +0.486. These averages include the correlations for alfalfa, which are, as shown by Table VIII, high for the crop of 1916.

Considering the correlations for oat straw, grain, and total crop on the several plots in 1917 and the yields of silage corn in 1918, we find that higher values of each of these measures of capacity for oat production are on the average followed by slightly, but perhaps not significantly, higher yields of silage corn in 1918 and generally by higher barley yields in 1919.

For the oat yields the average correlations with other crops are: for straw, +0.202; for grain, +0.289; and for total yield, +0.293.

The correlations of the yields of silage corn with the yields of the preceding crops are, with one exception, positive in sign. The average value for the eight years is +0.226.

The averages of the correlations between barley yields and the yields of other crops on the same plots during the eight years of the experiment are +0.141 for grain, +0.086 for straw, and +0.126 for total yield.

Summarizing this discussion of the results for the individual crops, we have the following average values of the correlation coefficients:

1911, sugar beets	−0.077	1915, ear corn	+0.167
1912, total alfalfa	+ .242	1916, ear corn	+ .486
1913, alfalfa I	+ .346	1917, oat straw	+ .202
1913, alfalfa II	+ .403	1917, oat grain	+ .289
1913, alfalfa I and II	+ .441	1917, total oats	+ .293
1914, alfalfa I	+ .401	1918, silage corn	+ .226
1914, alfalfa II	+ .354	1919, barley grain	+ .141
1914, alfalfa I and II	+ .407	1919, barley straw	+ .086
1914, alfalfa III	+ .366	1619, total barley	+ .126
1914, alfalfa I to III	+ .428	General average	+ .274

With the exception of the sugar beets the average correlation for every crop is positive in sign, and in many cases it is of a very material value.

Returning to the averages for the individual crops, we note from Table IX that the lowest correlation for alfalfa, whether with other cuttings of alfalfa or with the yield of other crops, is that for the single cutting of 1912.

It might be suggested that the 1912 yields of alfalfa are less likely to reflect the real producing capacity of the plots than the yields of the later cuttings of this crop, for the reason that the first cutting of alfalfa when sown without a nurse crop is subject to much variation because of slight differences in surface condition of the soil at seeding time and also because of differences in weediness of different plots. Both these conditions would become relatively less important in their effect on crop yield after the first cutting.

Because of its nitrogen-fixing capacity and the resistance to decay of the roots and stubble of alfalfa the correlation between the various yields of this legume and the yields of subsequent crops is of especial interest. Fortunately two crops of ear corn were grown immediately after the alfalfa, which was broken up in the fall of 1914.

A comparison of the correlations of these two series of corn yields with the preceding yields of alfalfa is made in Table X. These coefficients indicate a positive correlation between all the yields of alfalfa and the yields of ear corn in both 1915 and 1916.

Of the 19 correlations determined between the yields of alfalfa for 1912 to 1914 and the yields of ear corn in 1915 only 9 may be looked upon as probably significant in comparison with their probable errors. Of the 19 correlations between the yields of alfalfa in 1912 to 1914 and the yields of ear corn in 1916 only one coefficient—that for the 1912 yield of alfalfa and the 1916 yield of corn—can not be considered as representing a real agronomic relationship between yield of alfalfa and yield of corn.

The constants for 1916 are without exception larger and with two exceptions significantly larger in comparison with their probable errors than those for 1915.

TABLE X.—*Comparison of correlations between yields of alfalfa and yields of the first and of the second subsequent crop of corn*

	Whole plots.			Half plots.			Quarter plots.		
	1915	1916	Difference.	1915	1916	Difference.	1915	1916	Difference.
1912, alfalfa	+0.106±0.098 +1.08	+0.170±0.097 +1.75	+0.064±0.138 +0.46						
1913, alfalfa I	+.034±.099 +.34	+.647±.058 +11.2	+.613±.115 +5.34	+0.125±0.069 +1.80	+0.686±0.037 +18.4	+0.561±0.078 +7.17			
1913, alfalfa II	+.255±.093 +2.74	+.720±.048 +15.0	+.465±.105 +4.45	+.229+.067 +3.43	+.618±.043 +14.2	+0.389±.080 +4.89	+0.193±0.048 +4.04	+0.512±0.037 +14.0	+0.319±0.061 +0.53
1913, alfalfa I and II	+.168±.097 +1.73	+.801±.036 +22.3	+.633±.103 +6.12	+.208±.067 +3.09	+.768±.029 +26.6	+0.560±.073 +7.67			
1914, alfalfa I	+.189±.096 +1.97	+.800±.036 +22.2	+.611±.103 +5.96	+.228±.067 +3.42	+.744±.031 +23.7	+0.516± 074 +0.99	+.174±.048 +3.61	+.642±.029 +22.0	+.468±.056 +8.36
1914, afalfa II	+.041±.099 +.41	+.789±.038 +20.8	+.748±.106 +7.06	+.112±.069 +1.61	+.739±.032 +23.2	+0.627±.076 +8.25	+.109+.049 +2.22	+.629±.030 +21.0	+.520±.057 +9.06
1914, alfalfa I and II	+.120±.098 +1.22	+.858±.026 +33.0	+.738±.101 +7.29	+.185±.068 +2.72	+.820±.023 +35.7	+0.635±.072 +8.86	+.158±.048 +3.27	+.719±.024 +30.0	+.561±.054 +10.5
1914, alfalfa III	+.329±.088 +3.71	+.710±.049 +14.4	+.381±.101 +3.78						
1914, alfalfa I to III	+.191±.095 +2.00	+.880±.022 +39.4	+.689±.098 +7.07						
Average	.1592	.7083	.5491	.1811	.7291	.5480	.1585	.6255	.4670

16916—20——2

The average value for the nine pairs of correlations deduced from the yields of whole plots is +0.159 for alfalfa and corn yield in 1915 but +0.708 for alfalfa and corn yield in 1916. For the six pairs of correlations which may be deduced for half plots the average of the coefficients for the various yields of alfalfa in 1913 and 1914 and the yield of ear corn in 1915 is +0.181, whereas the average correlation of the same yields of alfalfa with corn one year later is +0.729. Finally, in the four cases in which it was possible to calculate correlations between alfalfa and corn yields on the basis of data for quarter plots the average for the correlations with ear corn in 1915 is +0.159, whereas the constants showing the relationship between the yield of alfalfa in 1913 and 1914 and ear corn in 1916 give an average of +0.626.

This more intimate relationship between the yields of alfalfa and the second crop of ear corn does not necessarily mean that the corn crop of 1916 was larger than that of 1915 but merely that the variations in the individual plot yields in 1916 are more dependent than those of 1915 upon the yields of alfalfa during 1912 to 1914. As a matter of fact the average yield in 1915 was 522.6 pound per plot, while in 1916 it was 396.2 pounds per plot. The greater yield in 1915 may have been, and probably was, due to factors other than soil conditions as such.

It is of interest in this connection to turn back to the table of coefficients of variation of yield (p. 347) and to note that for whole plots, half plots, and quarter plots the coefficients of variation of plot yield are distinctly lower in 1915 than in 1916. This result is quite in line with what one would expect if the fixed nitrogen of the varying growths of alfalfa were not yet fully available in 1915.

There is also another possible explanation for the lower correlation between the alfalfa yields and the yields of corn in 1915. It is always a difficult matter on the heavy soils at Huntley to break up alfalfa sod and to get the soil into good tilth for the succeeding crop. It may be that some of the plots in this field include heavier soil which ordinarily gives good yields but which was harder to get into good condition in time for the 1915 corn crop. If this were the case, these differences in tilth might have been smoothed out by the season's cultivation so as not to be expressed in the 1916 crop yields.

Some light may be thrown upon the problem of the residual influence of alfalfa in the following manner.

If the correlations between the plot yields of later crops be in a large degree determined by differences in fertility referable to differences in stand and yield of the preceding alfalfa crops, one might expect a closer correlation between the yields of ear corn in 1916 and oats in 1917 than between ear corn in 1916 and ear corn in 1915, since, as is shown above, variations in the alfalfa yields have little influence until 1916. This will be true, provided there be a residual influence of the variations in the yields of alfalfa such that these variations in fertility due to varia-

tions in yield from 1912 to 1914 inclusive will influence not merely the yield of corn in 1916 but the yield of oats in 1917, etc. The correlations between corn yields in 1915 and corn yields in 1916 and the yields of subsequent crops are shown side by side in Table XI.

TABLE XI.—*Comparison of correlations of the yields of ear corn in 1915 and in 1916 with the yields of subsequent crops*

	Corn, 1915.	Corn, 1916.	Difference.
1917.			
Oat grain	−0.025±0.099	+0.497±0.075	+0.522±0.124
Oat straw	+.112±.098	+.220±.095	+.108±.136
Total yield	+.072±.099	+.407±.083	+.335±.129
1918.			
Silage corn	+.459±.078	+.439±.080	−.020±.112
1919.			
Barley grain	+.042±.099	+.104±.098	+.062±.139
Barley straw	+.184±.096	+.144±.097	−.040±.136
Total yield	+.119±.098	+.135±.097	+.016±.138

These comparisons show that the yields of oats in 1917 are much more closely correlated with the yields of corn in 1916 than with the yields of ear corn in 1915. No such relationship is apparent in the correlations for silage corn in 1918 or for barley in 1919. The after effect of the alfalfa crops of 1912 to 1914 is, therefore, apparently largely limited to an influence on the yield of oats in 1917.

Turning from this indirect to a more direct method of comparison, we have determined the averages of the correlations between the several individual cuttings of alfalfa and the yields of the single antecedent and of the five subsequent crops. The results are given in Table XII.

TABLE XII.—*Averages of the correlations between the cuttings of alfalfa in 1912 to 1914 and the antecedent and succeeding crops*

Crop correlated with alfalfa.	Grain.	Straw.	Total yield.
Sugar beets, 1911	.....	.....	−0.082
.	.	.	.
.	.	.	.
.	.	.	.
Ear corn, 1915	+0.159	.....	.....
Ear corn, 1916	+.708	.....	.....
Oats, 1917	+.437	+0.210	+.371
Silage corn, 1918	.....	.....	+.279
Barley, 1919	+.234	+.113	+.195

There should be no correlation between the yield of sugar beets and alfalfa except that due to the initial heterogeneity of the field. The

insignificant negative correlation observed may be due to some peculiarity of the crop. The comparison of the correlation for the 1915 and 1916 corn crops has already been made (Table XI). Inspection of the averages in Table XII shows that on whatever character they are based the correlations decrease from the maximum relationship observed in 1916 to the lowest values in 1919.

Whether the residual influence of alfalfa *per se* has any influence on the 1919 or later crops can only be determined by further experimentation in which the interannual correlations can be deduced from the yields of plots upon which alfalfa has not been grown.

III.—DISCUSSION AND RECAPITULATION

The purpose of this paper has been to present the results of a new method of attack upon the problems of (*a*) the permanency of the differences which are found in the plots of an experimental field, and of (*b*) the influence of variations in the yields of certain crops in the rotation upon the yields of subsequent crops.

The data upon which the studies were primarily based comprise the yields of 46 plots—subdivided in several cases into half plots and quarter plots—each of 0.17 acre in area at the Huntley (Mont.) Field Station of the Office of Western Irrigation Agriculture for the nine years between 1911 and 1919, inclusive.

The uniform cropping experiment, involving sugar beets, alfalfa, corn, oats, and barley, was initiated merely to determine the variation in the yields of plots of a given size when homogeneously planted and uniformly treated. The experimental procedure was, therefore, determined in advance and was wholly independent of the statistical analysis. This is in certain regards fortunate. It frees the data absolutely from any suspicion of an influence of preconceptions or of personal equation on the biometric results. On the other hand, it is quite possible after the statistical analyses have been made to recognize ways in which the experiments could have been improved and made to yield more valuable results. This is, however, a feature of research in general. The discovery of inadequacies in a first set of experiments makes possible their elimination in subsequent work. The most unfortunate defect in the data was that the harvesting and weighing could not be done by half and quarter plots in 1917, 1918, and 1919, but this curtailment could not be avoided under existing conditions.

The results of a previous study (*3*) have shown that fields selected for plot tests of all kinds are practically without exception heterogeneous to a degree that influences profoundly the yields of the crops grown upon them. It was there pointed out that the correlation between the yields of adjacent plots might either be due to initial physical and chemical differences in the soil or be referable to the influence of previous crops upon the composition, texture, or tilth of the soil.

The first purpose of the present study has been to determine whether such differences in fields selected for their apparent uniformity by skilled agronomists are of a purely transitory nature or whether they are of a relatively permanent character.

This problem can be solved by determining whether in such series of uniformly treated plots the yields of the same plots in different years are correlated.

The results of the present study show that of the 152 correlations between the yields of the plots in different years, 133 are positive as compared with 19 which are negative in sign. The average value of the positive correlations is + 0.335, whereas the average of the negative constants is − 0.148. The general average is + 0.274. With the exception of the 1911 crop of sugar beets the correlation between the yields of each individual crop and the yields on the same plots in the eight other years of the experiment are on the average positive.

The data available for half and quarter plots fully substantiate the results for whole plots.

The results show conclusively, therefore, that plots, even of the small size and apparent uniformity of those at the Huntley Station, are characterized by differences which may persist throughout a period of years. Thus, in general, plots which produce more in one year will produce more in another year.

This is, of course, a well-recognized principle for large tracts. Its validity for small plots has apparently not been recognized heretofore. It is probably not a principle of universal applicability, because of the fact that meteorological as well as soil conditions play a large part in determining yield. It is quite probable that certain soil characteristics would result in maximum yields with one set of meteorological conditions but in minimum yields with another complex of aereal conditions.

The determination of the proximate factors to which these correlations are due presents a problem of considerable difficulty. Unfortunately (for this phase of the problem only) alfalfa was introduced early in the rotation and occupied the ground for three of the nine years covered by the experiment. It seems quite possible that the correlations between certain of the yields is due in part to the variation in nitrogen content of the soil referable to the variation in thickness of stand and strength of growth of the alfalfa crops.

The results show that there is but little correlation between the alfalfa yields of 1912 to 1914 and the ear corn yields of 1915, whereas the correlations for ear corn in 1916 are high. Thus the influence of alfalfa upon the yield of a subsequent crop is not fully evident until the second year after it is turned under.

There is a definitely demonstrable residual influence of the variation of alfalfa yields upon the yields of subsequent crops. The influence of

the alfalfa upon the yield of subsequent crops decreases with the lapse of time from the maximum correlation found for ear corn in 1916. The residual influence of the alfalfa is clearly marked in the oat crop of 1917 and may still be evident in the silage corn and barley crops of 1918 and 1919.

In view of the early introduction of alfalfa into the rotation, it is impossible to determine whether the correlations between yields other than those for alfalfa are due to the variation from plot to plot of the amount of nitrogen fixed by the alfalfa or whether it is to a considerable extent due to the original heterogeneity of the plots. This and other problems which will suggest themselves to the reader can be solved only by the analysis of further experimental data. The illustrations of the present paper are sufficient to show the value of the application of the interannual correlation method to agronomic problems.

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SOME CHANGES IN FLORIDA GRAPEFRUIT IN STORAGE[1]

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INTRODUCTION

Zoller (*11*),[3] in his paper on the constituents of the grapefruit (*Citrus decumana*), has pointed out that very little attention has been paid to the chemical constituents of this important fruit. This statement might also be made concerning the physiology of the fruit and the changes which go on in it after it is removed from the tree and held at storage temperatures. Some analyses of grapefruit have been made, however, by various investigators.

Chace, Tolman, and Munson (*4*) in their work on tropical fruits analyzed several different varieties of grapefruit. Rose (*8*) and others connected with the Florida Agricultural Experiment Station have made many analyses of citrus fruits in working out a basis for the regulation of the shipping of them. These last-mentioned analyses were for the most part determinations of the acid and sugar content of the pulp or juice and of the soluble solids present in the juice.

Collison (*5*) determined the acids and sugars in the juice of several varieties of grapefruit picked at various times during the season. He found, in general, that there was a decrease in acidity and an increase in sugar content as the season advanced and that after the fruit matured the sucrose was gradually broken down to reducing sugars. The fruits were analyzed shortly after removal from the tree.

Shamel (*9*) quotes a number of analyses of Florida and California grapefruit by E. M. Chace. Zoller (*11*) found that the acid of the pulp decreased during storage and records a marked increase in sugars after the fruit is removed from the tree. He found also that the content of the glucoside naringin, which is the bitter principle of grapefruit, was less in the peel after storage. This writer apparently used only a small number of fruits in his storage experiments, the work being for the most part a chemical study of the various constituents of the fruit.

Chace and Church (*3*) recently made a chemical study of some different types of grapefruit grown in California and Arizona. They determined

[1] This paper gives the result of a portion of the work carried on under the project "Factors Affecting the Storage Life of Fruits."

[2] The writers' thanks are due Mr. L. B. Scott, formerly Pomologist, Office of Horticultural and Pomological Investigations, for advice and helpful criticism while this work was in progress.

[3] Reference is made by number (italic) to "Literature cited," p. 372–373.

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the acid-solids ratio of grapefruit picked at intervals throughout the season from a number of localities. Some little work was also done on the effect of cold storage and storage in lemon curing rooms on the acid-solids ratio of the juice as compared to that of similar fruit direct from the tree. The data given seem to show that there is an increase in the acid-solids ratio during storage.

While other investigations have been carried out on certain chemical phases of the composition of grapefruit, the articles mentioned above are apparently all that are of interest in connection with the present work.

It is evident from the brief review of the literature here presented that the longer the fruit is held on the tree the lower the acid content. The acid content also apparently decreases during storage. The sugar content increases in fruit on the tree as the season advances, and some evidence is brought out that it increases during storage.

The present investigation was taken up to determine the effect of storage at various temperatures on the fruit and particularly on the sugar and acid content of the pulp, since these substances make up the major portion of the dry matter of the pulp or interior of the fruit, without the seeds.

METHODS OF EXPERIMENTATION

The fruit used in these experiments was from single trees of two named varieties, Silver Cluster and Davis, and "common Florida."[1] Most of the work was done with the two varieties last mentioned, the fruit of these varieties all being from three trees, one Davis and two "common Florida."[2]

The fruit from each tree was packed separately and was shipped to Washington, where the storage experiments were carried out. The first season's experiments, those of 1917–18, were preliminary, and only Silver Cluster fruit was used. All the fruit was obtained from one tree. It was shipped to Washington, where part of it was stored at 86° F. and the rest placed in a commercial cold storage at 32°. In the experiments with this fruit, the juice alone was analyzed, though the comparative percentage of peel and pulp was determined in some cases. The method followed was to peel the fruit, grind the pulp, and press out the juice through thin muslin. The acid-solids ratio was determined according to the usual method (*8*), and samples were, in most cases, made for sugar determinations. The samples for sugar determinations were pipetted into 250-cc. volumetric flasks, cleared with neutral lead acetate, made up to volume, filtered, and the excess lead removed with sodium oxalate. The reducing substances in this solution were determined. For total

[1] The writers are indebted to Mr. W. J. Krome, of the Medora Grove, Homestead, Fla., for his kindness in picking, packing, and shipping the fruit from these three trees at various times during the season, and to Mr. F. S. Poole, of Lake Alfred, for the Silver Cluster fruit used in the first season's work.

[2] "Common Florida" is the name applied in Florida to fruit of seedling grapefruit trees or trees budded from seedlings to which no distinctive varietal name has been applied. The term, therefore, may include fruit which represents a rather wide range in some of its characteristics.

sugars a 50-cc. aliquot was pipetted into a 100-cc. volumetric flask, the sucrose inverted by adding 5-cc. of concentrated hydrochloric acid and allowing it to stand overnight at room temperature. This solution was made up to volume, neutralized, and the reducing substance in it was determined.

Matthews's modification of Bertrand's method (*7, p. 994*) was followed in the determination of the sugars. The sugars were calculated as dextrose according to Munson and Walker's tables (*10*).

PRELIMINARY EXPERIMENTS, 1917–18

Table I shows the results obtained from several experiments in which fruit was placed in the incubator maintained at 86° F. In these experiments a sample consisting of six or more fruits was analyzed when the fruits were placed in the incubator, and analyses were made at the dates indicated in the first column. In experiment 2 fruit of the same lot as that used in experiment 1, which had been kept in cold storage since November 28, was placed in the incubator on January 6. The analyses on this latter date give data as to the effect of storage at 32° on the acid and sugar content of the fruit. The change in the acid-solids ratio of this fruit maintained at 86° for 15 and 28 days is shown in the table.

TABLE I.—*Changes in the composition of Silver Cluster grapefruit during storage at 86° F. as indicated by the change in acidity and sugar content of juice*

EXPERIMENT 1

Date sampled.	Acid as citric.	Soluble solids (Brix).	Acid-solids ratio.	Sugar as dextrose.		
				Reducing.	Sucrose.	Total.
	Per cent.			*Per cent.*	*Per cent.*	*Per cent.*
Nov. 28, 1917	1.16	9.15	7.9 : 1	2.95	2.76	5.71
Dec. 8, 1917	1.14	9.81	8.6 : 1	2.98	2.79	5.77
				3.03	2.59	5.62
Jan. 5, 1918	1.09	10.97	10.06 : 1	3.49	2.28	5.77

EXPERIMENT 2; FRUIT PLACED IN INCUBATOR

Jan. 6, 1918	1.14	8.95	7.8 : 1	2.90	2.68	5.58
				2.87	2.59	5.46
Jan. 21, 1918	.83	9.83	11.8 : 1	.....	.....	.....
Feb. 3, 1918	.76	9.29	12.2 : 1	.....	.....	.....

From Table I it is evident that there is a decrease in acidity when the fruit is stored at warm temperatures, while there is little, if any, decrease in the total sugar content of the juice. The reducing sugar is increased somewhat, but there is a corresponding decrease in the cane

sugar. The acid-solids ratio increases markedly in storage at 86° F., but there is no evidence of change at 32° in 38 days.

Some idea of the shrinkage in grapefruit and the change in acid-solids ratio was obtained in another experiment in which eight grapefruits which had been stored for 13 days were removed from storage, weighed, four of them peeled, and the percentages of peel and pulp determined. Acid, sugar, and soluble solids were determined in the juice of these four fruits. The other four fruits were placed in the incubator at 86° F. and allowed to remain 12 days. They were then removed, weighed, and the percentage of shrinkage, the percentage of peel, and the acid and soluble solids determined according to the usual method. The data obtained from these determinations are shown in Table II.

TABLE II.—*Acids, soluble solids, acid-solids ratio, shrinkage, and peel in single Silver Cluster grapefruit*

Fruit No.	When placed in storage.				Fruit No.	After 12 days' storage at 86° F.				
	Acid as citric.	Soluble solids (Brix).	Acid-solids ratio.	Peel.		Acid as citric.	Soluble solids (Brix).	Acid-solids ratio.	Shrinkage of fruit.	Peel.
	Per cent.			*Per cent.*		*Per cent.*			*Per cent.*	*Per cent.*
5.....	1.07	9.75	9.1 : 1	26	1.....	0.95	11.67	12.27 : 1	24	23
6.....	1.17	9.15	7.7 : 1	28	2.....	.93	11.61	12.49 : 1	32	18
7.....	1.12	9.09	8.1 : 1	27	3.....	1.00	11.07	11.07 : 1	26	21
8.....	1.14	10.35	9.1 : 1	27	4.....	1.02	11.61	11.37 : 1	35	18

The data in Table II show that the acidity decreases markedly and that the acid-solids ratio is much higher after storage for 12 days at 86° F. Much of this apparent increase in soluble solids is probably due to a concentration of the juice by the loss of water from the fruit. Inasmuch as the average shrinkage of the fruit is 29 per cent, while the average percentage of peel dropped from 27 to 20 per cent, obviously much of the water given off comes from the pulp.

EXPERIMENTS IN 1918–19

In the second season's work, Davis and "common Florida" grapefruits were obtained from Mr. W. J. Krome, Homestead, Fla. The entire crop from three trees was used in the storage experiments, one picking being made from the Davis tree and two from the "common Florida" trees. The fruit was shipped to Washington by express and stored at the cold-storage plant at Arlington Farm. Cold-storage temperatures of 32°, 36°, and 40° F. were used as well as common storage at a mean temperature of about 50°, probably fluctuating 5° above and below that temperature, and two warm storage temperatures which were about 70° and 86°, respectively. In most cases the fruit was weighed when placed in storage so that the skrinkage could be determined.

The structure of citrus fruit makes the study of the physiological changes taking place in it rather difficult. Considering the peel, pulp, and seeds of the fruit, there are then three structures which have very different water contents and water-holding powers. It is impossible to grind the entire fruit and weigh out comparable samples. It would be impossible to slice the fruit and expect the various slices to be comparable because of loss of juice from the pulp in slicing and the fact that the seeds are not necessarily evenly distributed. If fruits are sliced and the seeds removed, the operation is liable to be attended with a considerable loss of juice. After a number of experiments, the following method of sampling was decided upon. After the fruit was weighed it was peeled by making two cuts through the skin completely around the fruit, the cuts crossing each other at right angles at the stem and blossom ends. The peel was removed, and the thickness of each quarter was measured midway along the side by means of callipers. Such portions of the rag as adhered to the fruit were removed, and the fruit was weighed again. The percentage of peel was calculated from the weights before and after peeling. The fruit was divided into segments, and the seeds were removed, care being taken that no appreciable amount of juice was lost. Duplicate samples were made from segments from opposite sides of the fruits. One segment from each of the 10 fruits was used for each sample. While this method is not the most accurate, the results of analyses of duplicate samples indicate that it is sufficiently accurate for the work. It must always be taken into account that no two grapefruits have precisely the same chemical composition and that while in this work lots of 10 fruits were commonly used in each set of analyses, some variation will occur between any two lots no matter how carefully the fruits are selected.

In preparing the samples for analysis, the samples for sugar determinations were placed in beakers and covered with 95 per cent alcohol. A few drops of ammonia were added to neutralize the acidity, and the sample was brought to a boil. It was then transferred to extraction thimbles, the alcohol extract was separated at the same time by filtration, and the residue was subjected to continuous extraction for about 14 hours with alcohol in a soxhlet apparatus. The extract was added to the filtrate, the whole was made up to 1,000 cc. in a volumetric flask, and two 50-cc. aliquots were pipetted off for analysis. Sugar determinations were made according to the method already described.

For the acid determinations, the pulp was brought to a boil in water and was placed in liter volumetric flasks under toluol and allowed to stand with frequent shakings for about 10 days. It was then strained through linen, and two aliquots were titrated against sodium hydroxid, using phenolphthalein as an indicator. The dry-weight determinations were made by covering the samples with 95 per cent alcohol, driving off

the alcohol on a steam bath, and drying in a vacuum oven until there was no appreciable loss in weight between successive weighings. The results of the sugar, acid, and dry-matter determinations were calculated to percentage of wet weight of pulp. The percentage of peel was determined by weighing before and after peeling.

COLD AND COMMON STORAGE

As mentioned earlier in this paper, two pickings were made from the two "common Florida" trees, while all the fruit from the Davis tree was picked at the same time as the first lots from the other two trees. The first fruits were harvested October 31 and, as the cold-storage rooms were not yet completed, were allowed to remain in common storage at mean temperature of about 55° F. until November 21, when they were sampled. The fruit was then placed in the various storage chambers. The results of the analyses of the fruit held at 32°, 36°, 40° at various times during the storage season appear in Tables III and IV. The time in days after they were first sampled, when they were placed at the various storage temperatures, is given in the first columns, and the percentage of acid, sugar, dry matter, and the shrinkage of peel and percentage and thickness of peel appear in order. The second lots of fruit from the two "common Florida" trees were picked November 26, and the fruit was placed in the three cold-storage chambers December 4. Some of this picking was also placed in common storage, and the results of analyses of the fruit held in this type of storage are included with the data from the three cold-storage temperatures in Table III.

An inspection of Tables III and IV shows that there is a general decrease in tritratable acids during storage. This decrease would be more marked if it were possible to take into account the shrinkage of the fruit in storage. The actual decrease in acid would be somewhat more than that shown in the table.

In comparing the acid content of the fruit held at the three different cold-storage temperatures, 32°, 36°, and 40° F., it is evident that there is no constant difference in the rate at which the acid decreased. In most cases, however, at comparable samplings the fruit from the 40° storage is somewhat lower in acid content. This is especially noticeable in the Davis fruit (Table IV), where the fruit from the 32° storage is in all four samplings higher in acid content than that fruit from the other two cold storages.

The "common Florida" fruit in common storage was in general lower in acid than comparable lots in cold storage, with the exception of the second sampling which was made 42 days after the fruit was placed in storage. There was undoubtedly a greater shrinkage in the fruit in common storage, as was evidenced by the fact that the peel was thinner and the percentage of dry matter increased in the latter part of the season.

TABLE III.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of "common Florida" grapefruit at various times during storage season*

TREE I, FIRST PICK; PLACED IN STORAGE NOV. 21, 1918

STORED AT 32° F.

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
When placed in storage	1.02 1.05	2.16	2.17	4.33	8.20 8.14	.....	24.8	.....
After 60 days	.93 .94	2.71	2.35	5.06	8.06	.....	24.2	6.13
After 102 days	.97 .94	2.72	2.15	4.87	8.26 8.01	4.7	23.4	5.64
After 178 days	.89 .89	2.84	2.14	5.06	8.40 8.53	8.0	24.5	4.50
STORED AT 36° F.								
After 102 days	0.94 .97	2.35	2.27	4.62	8.51 8.14	3.8	24.4	6.22
After 178 days	.88 .90	2.86	2.04	4.90	8.6	5.9	22.7	4.30
STORED AT 40° F.								
After 60 days	0.98 .97	2.76	2.20	4.96	8.9	2.2	24.0	5.31
After 102 days	.91 .97	2.49	2.30	4.79	8.42 8.54	4.1	21.2	5.30
After 178 days	.87	2.69	2.16	4.85	8.13 8.04	5.5	21.1	4.00

TREE I, SECOND PICK; PLACED IN STORAGE DEC. 7, 1918

STORED AT 32° F.

When placed in storage	1.28 1.23	2.61	2.82	5.43	8.90 8.78	.....	.....	5.56
After 61 days	1.02	2.84	2.49	5.33	8.79	3.1	24.8	5.87
After 109 days	1.07	2.91	2.33	5.14	9.20 9.16	4.5	23.0	5.40
After 165 days	.89 .90	3.26	2.65	5.91	9.48 9.77	8.5	24.5	4.60
STORED AT 36° F.								
After 109 days	1.00 .98	2.85	2.66	5.51	9.22	3.6	23.0	5.90
After 157 days	1.00 .98	3.39	2.76	6.15	9.44 9.91	5.8	23.0	4.00
STORED AT 40° F.								
After 61 days	1.06 .98	2.58	2.43	5.01	9.00	2.6	22.7	5.57
After 167 days	1.01 .96	3.01	2.66	5.67	9.04 9.44	6.6	19.6	3.90
COMMON STORAGE								
After 42 days	1.11 1.22	3.24	2.45	5.69	8.90 9.02	.....	24.5	5.33
After 121 days	1.02 1.09	3.29	2.45	5.74	9.20 9.16	.....	24.4	4.60
After 179 days	.92 .77	3.00	3.17	6.17	10.00	.....	24.6	4.60

TABLE III.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of "common Florida" grapefruit at various times during storage season*—Con.

TREE 2, SECOND PICK; PLACED IN STORAGE NOV. 21, 1918

STORED AT 32° F.

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
When placed in storage	1.03 1.11	2.44	2.32	4.76	8.36 8.20	.....	24.6	6.30
After 61 days	.92 .92	2.63	2.43	5.06	8.60 8.22	.....	24	5.98
After 104 days	.98 .94	2.76	2.44	5.20	8.86 8.97	5.7	23.9	6.20
After 179 days	.90 .89	2.89	2.40	5.29	8.70 8.60	6.5	26.3	4.30

STORED AT 36° F.

After 104 days	.97 .98	2.68	2.47	5.15	8.70 8.68	5.7	24.2	6.20
After 153 days	.89 .91	3.03	1.78	4.81	8.77	5.7	24.2	4.20

STORED AT 40° F.

After 61 days	.96	2.90	2.44	5.34	8.75	2.6	26.4	6.73
After 106 days	.92 .90	2.87	2.08	4.95	8.88 8.67	7.2	25.6	5.10
After 153 days	.89 .91	3.03	2.15	5.18	8.70 8.60	6.5	28.3	4.30

TREE 2, SECOND PICK; PLACED IN STORAGE DEC. 7, 1918

STORED AT 32° F.

When placed in storage	1.12 1.12	2.77	2.43	5.20	8.96 9.12	.....	24.8	6.08
After 62 days	.....	3.38	2.94	6.32	9.4 9.49	2.9	22.6	6.09
After 109 days	.94 .93	3.07	2.86	5.93	.....	5.1	24.1	5.90
After 169 days	.97 .94	3.06	2.90	5.96	9.12 9.84	7.8	19	5.00

STORED AT 36° F.

After 109 days	1.02 .99	2.99	2.70	5.69	9.59 9.28	4.8	.....	.....
After 157 days	.96	3.32	2.59	5.91	9.80 9.89	5.6	24.8	5.00

STORED AT 40° F.

After 62 days	0.94	.....	.....	.....	9.7 9.6	2.5	24.5	6.09
After 134 days	.93 .91	3.03	2.36	5.39	9.44 9.71	6.5	24.2	4.60

COMMON STORAGE

After 42 days	1.06 1.07	3.55	2.23	5.78	9.43	.....	24.3	.....
After 111 days	.94 .91	3.23	2.12	5.35	10.10 9.77	.....	22.7	4.74
After 179 days	.83 .80	3.56	2.28	5.84	9.84	.....	20.4	3.70

TABLE IV.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of Davis grapefruit at various times during storage season*

PLACED IN STORAGE NOV. 21, 1918

STORED AT 32° F.

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
When placed in storage	0.93 .96	2.69	1.66	4.35	7.94 8.10	.....	23.7	5.10
After 58 days	.91 .97	3.06	2.29	5.35	8.41 8	3.5	23.1	4.73
After 88 days	.86	3.05	2.09	5.14	8.37	3.9	23.4	5.16
After 118 days	.83 .83	3.25	2.02	5.27	8.23 8.09	3.3	25	4.8
After 140 days	.84 .84	3.09	2.03	5.22	8.91	5.5	26.5	5.6
STORED AT 36° F.								
After 58 days	0.87	3.11	2.23	5.34	8.43 8.42	2.6	23.5	4.72
After 88 days	.79 .76	3.10	2.14	5.24	8.39 8.25	5.3	22.1	4.56
After 118 days	.80 .75	3.00	2.10	5.10	8.27 8.69	6	22.1	4.7
After 139 days	.78 .80	3.16	2.73	5.99	8.02 8.26	8.7	22.5	4
STORED AT 40° F.								
After 58 days	0.85	3.62	2.04	5.66	8.53	1.2	23.3	4.7
After 88 days	.79	3.05	2.10	5.15	8.5 8.41	7.6	19.9	4.01
After 118 days	.77 .73	3.14	2.09	5.23	8.43 8.13	8.1	18.9	3.9
After 139 days	.73 .75	3.18	2.14	5.31	8.47	7.5	19.2	3.9

A comparison of the acid content of the fruit from the two different pickings, when placed in storage, showed that the fruit picked last has a somewhat higher acid content, probably because the fruit of the first picking stood in common storage 22 days before the first analyses.

The sugar content of stored fruit is in rather striking contrast to the acid content. With few exceptions, the percentage of total sugar is higher in the stored fruit than in the samples analyzed when the fruit was placed in storage. In some cases, as in the Davis fruit (Table IV), which had been stored 139 days at 36° F., the sugar content is more than 30 per cent higher than in the analyses made when the fruit was placed in storage. The difference is as marked in other cases. In general, however, the increase in total sugar content is more apparent than real and is probably due to the loss of water from the fruit. The shrinkage of the fruit is in many cases sufficient to account for the apparent increase in sugar content. It is, however, undoubtedly true that there is no appreciable diminution of the sugar content during storage at the four temperatures here considered.

The sucrose content, when calculated as percentage of pulp, remains about the same during storage. Apparently the breaking down of the sucrose just about keeps pace with the shrinkage of the fruit. This

increase in total sugars, then, as the storage season advances, is due to an increase in free-reducing substances.

The dry-matter determinations are not particularly conclusive in the analyses here shown. A careful inspection of the data obtained from the 17 storage experiments shown in Tables III and IV indicates that there is, in general, an increase in dry matter. This is probably due to the loss of water from the fruit as well as to losses from respiratory activities, both of which are included in shrinkage.

The shrinkage increases with the length of time the fruit remains in storage and is in general around 5 per cent for the first 100 days in cold storage. Only in two cases is it more than 8 per cent for the entire time the fruit was stored. There is no marked difference in shrinkage in the three temperatures. That the shrinkage is from the pulp as well as the peel is shown by the fact that the decrease in the percentage of peel is not sufficient to account for the loss in weight.

In general, the peel is from 19 to 25 per cent of the fruit used in these experiments, and there is no wide variation between the two varieties. The decrease in thickness of the peel during storage is about 30 per cent, due, probably for the most part, to loss of water.

WARM STORAGE

As mentioned in the earlier part of this paper, in addition to the three cold-storage and one common-storage temperatures, grapefruits were placed in two warm storages at temperatures of about 70° and 86° F. Some lots of fruit were stored in boxes and others in lard cans with tight-fitting lids, the lids being removed from the cans occasionally for a short time to aerate the fruit. The storage season for this fruit was, of course. not so long as for that stored in the cold- or common-storage temperatures, The results of analyses of fruit stored at 70° are shown in Table V, while data obtained from the 86° storage are given in Table VI.

TABLE V.—*Percentage of sugars, acids, dry matter, peel, and thickness of peel of "common Florida" grapefruit stored at about 70° F. in ventilated and unventilated packages*

TREE 1, FIRST PICK

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.			
							Mm.
When placed in storage.....	1.02 1.05	2.16	2.17	4.33	.....	24.8	.....
After 61 days, unventilated.....	.98 1.03	2.17	1.82	4.69	8.68 8.47	.....	6.01
TREE 2, FIRST PICK							
When placed in storage.....	1.03 1.11	2.44	2.32	4.76	8.36 8.20	24.6	6.00
After 50 days, unventilated.....	1.02 1.01	2.96	1.95	4.91	8.9 8.45	24.5	6.13
After 50 days, ventilated.....	.94 .93	2.98	2.40	5.38	9.90	17.12	3.03

TABLE VI.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of grapefruit stored at about 86° F. in ventilated and unventilated packages*

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
TREE 1, "COMMON FLORIDA," FIRST PICK								*Mm.*
When placed in storage	1.02 1.05	2.16	2.17	4.33	8.20 8.14	.....	24.8	.....
After 30 days, unventilated	1.04 1.12	2.77	2.01	4.78	9.12 9.30	.....	24.6	5.77
After 30 days, ventilated	1.26 1.19	2.88	2.09	4.97	9.01 9.33	.....	20	3.12
TREE 1, "COMMON FLORIDA," SECOND PICK								
When placed in storage	1.28 1 23	2.61	2.82	5.43	8.90 8.78	.....	.....	5.56
After 60 days, ventilated	1.25 1.10	.....	.....	.....	10.44	26.9	12.5	2.41
After 86 days, ventilated	1.16 1.18	3.98	2.02	6.00	12.09	34.6	11.5	2.08
TREE 2, "COMMON FLORIDA," FIRST PICK								
When placed in storage	1.03 1.11	2.44	2.32	4.76	8.96 9.12	.....	24.6	.....
After 30 days, unventilated	1.14 1.12	3.28	2.02	5.30	.....	.....	24.3	5.95
TREE 2, "COMMON FLORIDA," SECOND PICK								
When placed in storage	1.12 1.12	2.77	2.43	5.20	8.96 9.12	.....	24.8	6.08
After 61 days, ventilated	.96 1.15	3.95	1.99	5.94	10.10 9.75	25.3	13.8	2.42
After 86 days, ventilated	1.26 1.20	4.32	2.23	6.55	12.23	34.7	11.4	2.64
DAVIS								
When placed in storage	0.93 .96	2.69	1.66	4.35	7.94 8.10	.....	23.7	.....
After 19 days, unventilated	.90 .96	2.88	2.32	5.20	8.33 8.21	.....	23.7	.....
After 24 days, ventilated	.94	2.78	2.29	5.07	9.76	.....	14.7	.....

In an inspection of the tables it may be seen that in general there is very little, if any, decrease in titratable acids in the fruit stored in cans, that is, in unventilated packages, at either of the two temperatures. In some cases there is an apparent increase, as in tree 1 of "common Florida," first pick (Table VI), which had been stored 30 days at 86° F. and again in tree 2 of the same variety, pick, temperature, and length of storage period. The increase in total sugar content is more, comparatively, in both these cases than is the increase in acid. In all other cases the fruit in the unventilated package has an acid content about

the same as when placed in storage and an increased sugar content. There is some loss of water from the fruit even in the cans which are closed most of the time, and it is possible that the acid decreases, the decrease in most cases being as rapid as the shrinkage of the fruit. It is, of course, always possible that at these high temperatures and under the low oxygen pressures some acid is formed in respiration.

With the stored fruit in ventilated packages the analyses made after 24 or 30 days, as shown in Table VI, gave an acid content as high as or higher than when the fruit was placed in storage. At the longer storage periods in both temperatures the acid content was usually lower than at the beginning of the storage period. In every case there was a marked increase in sugar content, as calculated to wet weight of pulp. This increase was greater where the fruit had been in storage more than 30 days.

While no exact data are at hand, it seems probable that the increase in acid is due, for the most part, to loss of water from the fruit. Cases in which the shrinkage was determined show that it was over 34 per cent in 86 days at 86° F., the higher storage temperature. The thickness of the skin of the fruit and the percentage of peel decrease markedly in ventilated warm storage. This, of course, makes impossible the calculation of the actual shrinkage of the pulp. The percentage of total sugar in the pulp is in all cases higher after storage. This increase is due in most cases to an increase in the reducing-sugar content, for the percentage of cane sugar remains about the same in all analyses. It is quite possible, in spite of the apparent increase in sugar content, that some of the sugar originally present in the fruit actually disappears during storage.

Another series of experiments was carried out in which fruit from the second picking of the two "common Florida" trees was placed in the warm room at 70° F. after it had remained in common storage 51 days. The fruit was stored in cans and boxes, as in the experiments just described. The results are given in Table VII.

From Table VI it is apparent that fruit removed from common storage and placed at a higher temperature behaves the same as fruit stored at the higher temperature throughout the season. The findings in this series are then mostly corroborative.

In ventilated packages there was, in some cases, an apparent increase in acids, and in others the acid content was a little less. If the exceedingly high percentage of shrinkage is taken into account, the results seem to indicate that there is no actual increase in the amount of acid during storage and that there may be a decrease as compared with the amount originally present. The sugar content of the fruit stored in unventilated packages shows always a decrease in the percentage of total sugars present, while in ventilated storage the increase in sugar content is in no case more than sufficient to account for the probable

shrinkage of the pulp. In one case, tree 1, stored 67 days, the sugar content is less after storage, probably because of variation in the samples. The results indicate that there may be a slight decrease in sugar at the higher storage temperatures.

TABLE VII.—*Percentage of sugar, acids, dry matter, shrinkage of fruit, peel, and thickness of the peel of "common Florida" grapefruit stored 51 days in common storage then placed in warm storage*

TREE 1, SECOND PICK

Time of sampling.	Acids as citric.	Sugars as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
After 42 days in common storage	1.11 1.22	3.24	2.45	5.69	.....	.....	24.5	5.33
After 51 days in common storage; 29 days at 70° F., ventilated	1.13	3.43	2.56	5.99	.....	10.8	20.5	3.57
After 51 days in common storage; 29 days at 70° F., unventilated	1.02 1.01	3.20	2.38	5.58	.....	1.2	23	5.76
After 51 days in common storage; 67 days at 70° F., ventilated	1.15 1.11	3.62	1.94	5.56	9.44 9.34	20.1	17.5	2.70
After 51 days in common storage; 67 days at 70° F., unventilated	.99 1.03	3.21	2.17	5.39	9.45 9.64	3.3	26.1	.....

TREE 2, SECOND PICK

After 42 days in common storage	1.06 1.07	3.55	2.22	5.78	9.43	.....	24.3	.....
After 51 days in common storage; 28 days at 70° F., ventilated	1.12 1.10	3.76	2.28	6.04	9.61 9.40	11	19.9	3.35
After 51 days in common storage; 28 days at 70° F., unventilated	1.02 1.03	3.32	1.99	5.31	10.1 10.41	1.1	22.8	4.88
After 51 days in common storage; 67 days at 70° F., ventilated	1.12	3.65	2.17	5.82	10.34 10.01	23	7.5	.50
After 51 days in common storage; 67 days at 70° F., unventilated	.99 .96	3.26	2.20	5.46	9.65 9.32	4.8	24.4	5.30

There is a marked difference in the shrinkage of the fruit and percentage of peel as well as in thickness of the peel in the ventilated and unventilated packages, the shrinkage being around 4 per cent in the unventilated fruit for 67 days and from 20 to 23 per cent for comparable lots stored in ventilated packages. The peel, as would be expected, becomes very much thinner in the fruit stored in ventilated storage.

There is a marked increase in the percentage of dry matter in the pulp of the fruit stored in ventilated storage, while that of fruit in unventilated packages remains practically constant.

To determine the effect of cold storage followed by warm storage upon the keeping quality of the fruit and also to obtain more data as to the acid-sugar changes, "common Florida" grapefruits of the first pick, which had been maintained at 32° F. for 61 days were removed, weighed, and placed in boxes at 70°. The analyses of this fruit after 46 days at 70°, as compared with the analyses of comparable lots from 32° at the time the fruit was placed in the warm chambers, are given in Table VIII.

TABLE VIII.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of "common Florida" grapefruit stored in cold storage 61 days and removed to warm storage for a period*

TREE 1, FIRST PICK

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
After 60 days at 32° F.	0.92 .93	2.71	2.35	5.06	8.06	.....	.....	6.13
After 61 days at 32°; 46 days at 70°, ventilated.	.99 .97	2.91	2.39	5.31	9.86 9.80	24.1	17.3	2.70

TREE 2, FIRST PICK

After 61 days at 32°..	0.92 .92	2.63	2.43	5.06	8.60 8.22	.....	.....	5.98
After 61 days at 32°; 46 days at 70°, ventilated.	1.01 1.05	3.18	2.19	5.37	9.24 9.32	17.4	21.1	3.10

It is evident from Table VIII that there is an apparent increase in acidity, as was the case in most of the other warm-storage experiments. The total sugar content is somewhat increased, though less proportionally than the acid content. The percentage of dry matter is increased markedly, the shrinkage at the high temperature is very marked, the percentage of peel decreases, and the peel becomes thinner, the fruit behaving much as in all the warm-storage experiments.

It seems probable that there was in these experiments a decrease in the sugar during the period of warm storage, while the amount of acids remained about the same. The fruit compared very favorably in analyses with the grapefruit from the warm-storage experiments, the results of which are given in Tables V to VII.

GENERAL DISCUSSION

While this investigation is primarily concerned with the acid and sugar changes in the fruit, some data were obtained as to the general appearance and attractiveness of fruit stored at the various cold-storage temperatures and also at common storage.

The fruit will apparently keep for a longer period in cold storage than in either common or warm storage. In the first place, the losses from decay caused by microorganisms are much less in the cold-storage temperatures. In the second place, the shrinkage in cold storage is much less than in warm, ventilated storage or in common storage. A high percentage of the fruit rotted in warm, unventilated packages. A high degree of humidity is necessarily maintained in this storage, which is very favorable to the growth of various fungi which break down the fruit. There is, therefore, much loss. The fruit which does survive this treatment is, however, very attractive in appearance and has an excellent flavor. In the third place, the life of the fruit is apparently lengthened in cold storage—that is, the average fruit apparently tends to break down more quickly when maintained at temperatures above 40° F. than when stored at lower temperatures.

An undesirable feature of cold storage is the breaking down or pitting of the peel at the temperature of 40° F. or lower. This breaking down of the peel begins as a slightly sunken spot, which increases in size and becomes brown in color. The sunken portions are usually not more than ½ inch in diameter, but several may coalesce, making a large sunken area of dark-brown tissue. This does not extend into the pulp, and the flavor is apparently unaffected, but the fruits are rendered unsightly. In these experiments no pitting was noticeable on the fruit stored at the two warm-storage temperatures or in common storage. It occurred only on the fruit stored in the three cold storages. In these temperatures the fruit at 40° was most seriously affected. There was somewhat less pitting on the fruit in the 36° storage and only a little on fruit at 32°.

The flavor of the fruit improves in cold storage. The fruit is sweeter, as is obvious from the fact that the sugar content of the pulp is higher and the acid content lower. The fruit is apparently not so bitter after storage, which may be due to the breaking down of the naringin in the pulp. Zoller (*11*) has shown that this glucoside breaks down in the peel during storage. The fruit improves in taste more rapidly at high storage temperature than in cold storage, which is to be expected, inasmuch as the changes are more rapid in warm storage. After longer storage, however, the fruit in cold storage attains the excellence brought about more quickly at a higher temperature.

The experiments in which the fruit was removed from storage at 32° F. after 60 days and stored at 70° for 46 days (Table VIII) indicate that the grapefruit does not deteriorate rapidly after removal from cold storage. The fruit compared very favorably with fruit that had been stored at 70° from the beginning of the storage period.

From the data shown in Tables I to IX, there is no question but that the titratable acids in the fruit decrease after the fruit is removed from the tree and placed in cold storage, which is in accord with the behavior

of the acids in apples, as found by Bigelow, Gore, and Howard (*2*), and others, Bigelow and Gore on peaches, (*1*) and in pears by Magness (*6*).

The sugar content apparently does not decrease appreciably in cold storage, though definite evidence on this point is lacking. The shrinkage of the peel and pulp may not be proportional, so that an accurate determination of the original weight of the pulp is impossible. There is indication that the sugar content decreases slightly in warm storage if the shrinkage of the fruit is taken into consideration. There was in no case evidence of a markedly increased sugar content in the fruit, mentioned by Zoller (*11*). There is considerable variation in individual fruits, and it is possible that this would account for the increase in sugar content which he found. In the preliminary experiments, the results of which are given in Tables I and II, it is shown that there is a marked increase in the acid-solids ratio after storage at 86° F. This increase in amount of soluble solids is undoubtedly due mainly to the loss of water from the pulp and a concentration of the juice. While acid-solids determinations were not carried out in the later experiments, the results of the sugar and acid determinations show that a similar condition would hold for fruit stored at the cold-storage temperatures, though possibly not for fruit stored for long periods at the higher temperatures used.

In conclusion, it has been shown in this investigation that the acid content of grapefruits decreases in cold storage. There is an apparent increase in sugar content in cold storage, calculated to percentage of pulp, which seems to be due to loss of water from the fruit. The dry matter increases during storage. The shrinkage of the fruit runs from 5 to 8 per cent in cold storage to around 23 per cent in warm, ventilated storage.

Fruit was kept in cold storage for about six months. The best storage temperature seemed to be 32° F, for at this temperature the pitting was much less marked. Pitting of grapefruit does not apparently develop at high temperatures but occurs only on the cold-storage fruit. Grapefruits do not keep so long in common storage or warm storage as in cold storage. There is much more loss from decay at the higher temperatures.

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A BACTERIOLOGICAL STUDY OF CANNED RIPE OLIVES

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As a result of the first of the recent series of outbreaks of botulism traceable to the consumption of ripe olives infected with *Bacillus botulinus*,[2] many lots of canned ripe olives were collected by inspectors of the Bureau of Chemistry for bacteriological examination. These were obtained, for the most part, from various retail and wholesale houses in all parts of the country and bore the label of the same company as did those responsible for the fatalities. While the primary object of the investigation was the detection of the presence of *Bacillus botulinus*, this object was extended to include a study of the types of microorganisms responsible for the spoilage and also to determine whether viable microorganisms might be encountered in apparently normal containers. The containers subjected to examination included all sizes of both cans and glass jars. Some were apparently normal while others were swelled or obviously spoiled.

In the bacteriological examination of these samples the following procedures were adopted as a routine. All containers were opened with usual aseptic precautions, and 1.5 to 2 cc. of the liquor were withdrawn by means of a sterile pipette. Approximately 0.5 cc. of this was spread over a dextrose agar slant (for aerobes), and the remainder was then run into a tube of infusion broth under oil. This medium was a 0.2 per cent dextrose beef infusion broth (P_H 7.4 to 7.6). It was covered before autoclaving with a layer of liquid petrolatum. In place of this medium there was occasionally used a 2.0 per cent dextrose-beef infusion broth, similarly covered with a layer of oil and containing a small piece of meat. In most cases a piece of olive was removed with sterile knife or forceps and was transferred to the dextrose broth tube. Incubation was at 37° C. In addition, notes were kept on the condition of the container, whether normal, swelled, etc., and also on the odor. Cans which were obviously leaking were discarded.

It is realized that for the sake of completeness it would have been desirable to have included a greater variety of culture media and several

[1] The author wishes to express his appreciation of the valuable criticism and suggestions given by Dr. Charles Thom, of the Microbiological Laboratory.

[2] ARMSTRONG, Chas., STORY, R. V., and SCOTT, Ernest. BOTULISM FROM EATING CANNED RIPE OLIVES. *In* Public Health Rpts., v. 34, no. 51, p. 2877–2905, 5 fig. 1919.

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temperatures of incubation. The number of samples, as well as the urgency of the examination, however, forbade any elaborate series of tests. The total number of cans and glass jars, both normal and spoiled, which were cultured by various members of this laboratory, together with the number showing the presence of living organisms, is summarized as follows:

Experiment with cans

Exp. No.	
I.	Number of normal cans cultured 181
	Of this number 173 were sterile, while 8, or 4.4 per cent, were found to contain viable microorganisms.
II.	Number of "swelled" or "springy" cans cultured 157
	Of these 154 contained living microorganisms, while 3 were apparently sterile (2 of these 3 were "springers," the other was a "hard swell").
	Total number of cans cultured 338

Experiment with glass containers

Exp. No.	
I.	Number of containers normal in appearance and odor 116
	Of this number 105 were sterile, while 11, or 9.5 per cent, revealed the presence of living microorganisms.
II.	Number of containers obviously spoiled or of bad odor 26
	All of these 26 gave positive cultural results.
	Total number of glass containers cultured 142
	Total number of cans and glass containers cultured 480

Thus, it is seen that all the obviously spoiled glass jars, and, with one exception, all the swelled cans revealed the presence of living microorganisms. On the other hand, the normal containers were, for the most part, sterile. In this connection it is interesting to note that 4.4 per cent of the normal cans were found to contain viable organisms, while in the normal glass containers the proportion was decidedly higher, namely, 9.5 per cent. Of the 157 swelled or "springy" cans, all but three gave positive cultural tests. Two of these three were "springers," due probably to imperfect exhausting, and were no doubt otherwise normal. The third was a "hard swell." Whether the failure to obtain living organisms from this one can was due to lack of a greater diversity of culture media or whether the causative organisms had been killed as a result of their own metabolic products is not known.

Of the total of 480 containers examined bacteriologically, 117 of those which had yielded positive cultural tests were studied further to gain some knowledge of the types of organisms present. As a rule, extensive cultural and biochemical tests were omitted, and merely the general type or group to which the organisms belonged was determined. A summary of the types obtained from the 117 containers thus studied is shown below. The figures indicate the number of times each was encountered.

Types of organisms found

Colon group	81
Colon group, sluggish liquefaction of gelatin (*Bacillus cloacae*)	4
Bacterium fluorescens (liquefying)	2
Proteus	3
Other Gram-negative, non-spore-forming bacilli, not identified	5
Gram-positive, aerobic, spore-forming bacilli, gelatin liquefiers—	
Bacillus cereus type	3
Bacillus mycoides type	4
Bacillus mesentericus type	6
Type not determined	19
Slender, Gram-positive, aerobic or facultative anaerobic bacilli, oval terminal spores, gelatin not liquefied	10
Gram-positive diplococci	31
Gram-positive staphylococci	10
Spore-forming, obligate anaerobes	6
Yeasts	3
Mold (*Aspergillus terreus*) [1]	1

In addition to these, *Bacillus botulinus* was found in 7 of the spoiled glass jars. A report of the findings of this laboratory with respect to *Bacillus botulinus*, both from the material obtained in the open market and from specimens received from the poisoning cases, has been made the subject of another paper.[2]

The large proportion of non-spore-forming organisms, particularly of the colon group and the Coccaceae, was indeed surprising. Many of the cultures of the colon group when first isolated exhibited a delayed fermentation of lactose somewhat similar to that reported by Bronfenbrenner and Davis,[3] though not so marked. In lactose broth, acid formation was delayed from 48 to 72 hours, while gas was produced only after 3 to 5 days' incubation. After several successive transplants in lactose broth, fermentation of this sugar was markedly accelerated.

Although some of the organisms obtained were placed without difficulty in their proper groups, others were not identified by the limited number of cultural tests employed, and these are designated in the foregoing list by their chief cultural characteristics or morphology. Many Gram-positive diplococci were encountered. These exhibited a distinct lance-shaped appearance in liquid media, with occasional short chains of three or four elements. On dextrose agar slants the individual colonies appeared as minute white pin points. In beef infusion broth under oil

[1] For identification of this species the writer is indebted to Dr. Margaret B. Church, of the Microbiological Laboratory.

[2] DeBord, G. G., Edmondson, R. B., and Thom, Charles. SUMMARY OF BUREAU OF CHEMISTRY INVESTIGATIONS OF POISONING DUE TO RIPE OLIVES. *In* Jour. Amer. Med. Assoc., v. 74, no. 18, p. 1220-1221, 1920.

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the growth was fairly luxuriant, producing a distinct cloudiness after 24 hours' incubation. The other type of Gram-positive coccus encountered grew more luxuriantly on plain agar slants and was found upon staining to occur in irregular clusters. The several obligate anaerobes were inoculated into milk and into the meat medium of Holman.[1] One culture digested the meat with a distinct putrefactive odor. The remaining five caused neither putrefaction of meat nor stormy fermentation of milk. Dextrose was attacked with acid and gas production. Up to the present time they have not been studied further.

FLORA OF SWELLED CANS.—The flora of swelled cans was found to consist largely of members of the colon group, for of 85 swelled cans studied this group was obtained from 75, and from 40 of these in apparently pure culture. In the others they were found in mixed culture with the several types of Coccaceae, the aerobic, Gram-positive, spore-forming bacilli, or, more rarely, with an obligate anaerobe, with Proteus, or with a yeast. In three instances spoilage, with resultant swelling of the can, was evidently due to spore-forming anaerobes only. In one instance Proteus was found in pure culture. A few of the swelled cans yielded cultural results from which no evidence could be gathered as to the type of organism causing gas formation within the can. Thus, an aerobic, spore-forming, Gram-positive rod was the only type obtained from 2 swelled cans, while from 2 others Gram-positive cocci were obtained in pure culture. Since none of these organisms attacked carbohydrates[2] with gas production, it is evident that the gas-producer had disappeared or was overlooked.

NORMAL CONTAINERS.—As previously shown, 8 normal cans and 11 normal glass containers were found to contain living microorganisms. Four of these 8 normal cans yielded cultures of the colon group. The others contained cocci and several types of aerobic, spore-forming bacilli. The finding of members of the colon group in 4 of the normal cans was rather surprising. Evidently for some unknown reason the bacilli failed to multiply to any extent in these cans. Without exception, the types encountered in the normal glass jars were aerobic, spore-forming, Gram-positive rods. Several were identified as *Bacillus mesentericus* and one as *Bacillus cereus*.

SPOILED GLASS JARS.—The flora of the spoiled glass jars was as a rule more varied and complex than that of the swelled cans. The contents of several jars were obviously spoiled and disintegrated to a mushy consistency with a disagreeable odor, unrecognizable as that of olives. These yielded a diversity of types of which the following are illustrative:

[1] HOLMAN, W. L. THE VALUE OF A COOKED MEAT MEDIUM FOR ROUTINE AND SPECIAL BACTERIOLOGY. *In* Jour. Bact., v. 4, no. 2, p. 149–155. 1919. References, p. 155.

[2] Chemical analyses by the Food Control Laboratory of the Bureau of Chemistry showed the liquor in which the olives were packed to contain from 0.16 to 0.23 per cent reducing sugars after inversion, expressed as percentage of invert sugar.

1. Putrefactive anaerobe which digested a cooked meat medium with a putrefactive odor, an aerobic Gram-positive, spore-forming rod, and an unidentified Gram-negative bacillus.

2. *Bacterium fluorescens liquefaciens*, Proteus, aerobic Gram-positive, spore-forming bacillus, and an unidentified non-gas-producing Gram-negative bacillus.

3. Staphylococcus, a yeast, and Gram-positive, sporing bacillus.

4. Gram-positive diplococci, colon group, *Aspergillus terreus*, and a Gram-positive, spore-forming rod.

No definite correlation between the odor of the spoiled samples and the type of organism contained therein was noted. The swelled cans from which the colon group only was obtained were recorded as possessing either a flat or slightly "off" odor—that is, they lacked the characteristic fragrant aroma of the first-class product. Since many of the sterile normal cans, particularly of certain brands, had a similar odor, it is doubted whether this condition can be ascribed solely to the metabolic activities of the colon group. Three cans containing spore-forming anaerobes possessed a disagreeable or rancid odor. The liquor, together with portions of the olives from several of the most offensive cans, was fed to guinea pigs without ill effects.

The large numbers and diversity of types encountered, particularly of the non-spore-formers, point to insufficient heating of the product. While it is realized that there may be a slight leakage along the seam of the can immediately after heating, and with subsequent closure, it would seem improbable that this could account entirely for the results obtained in this investigation.

SUMMARY

(1) In the bacteriological examination of 480 commercial containers of ripe olives, living microorganisms were obtained in practically every instance from samples which were abnormal, as indicated either by a swelled condition of the container or a bad odor.

(2) Viable microorganisms were found in a small percentage of normal containers. These were either aerobic, spore-forming bacilli, cocci, or apparently dormant members of the colon group.

(3) A study of the organisms encountered in the spoiled samples showed a great diversity of types, among which the colon group predominated.

RELATION OF THE SOIL SOLUTION TO THE SOIL EXTRACT

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Modern views of soil fertility recognize the general principle that plants derive their immediate supply of inorganic elements entirely from the soil solution. It has also been proved that the soil solution is subject to highly significant fluctuations. The concentration and composition of the soil solution may undergo very great alterations as a result of seasonal changes, crop growth, activities of microorganisms, rainfall, fertilization, etc. The evidence supporting this point of view is now too strong to admit of any doubt. It is justifiable to assume, therefore, that further progress in the study of the soil as a medium for plant growth will depend upon an increased knowledge of the soil solution, particularly in its dynamic relations to the soil mass, to the plant and microorganisms, and to the application of fertilizing materials.

Experimental work on the soil solution immediately encounters a formidable obstacle in the difficulty of separating from the soil the solution which provides nutriment to the plants. When the soil contains moisture in percentages most suitable for plant growth, the solution is held by the soil particles with such force that no ordinary means will serve to effect a separation. This fact is well recognized, and various attempts have been made to overcome the difficulties involved and to obtain the soil solution in an unmodified state. The most important developments in this phase of the work have been described by Morgan (*8*) [1] and by C. B. Lipman (7). It is yet too early to state any final conclusions based on data obtained by these methods, but their further perfection may lead to the attainment of most essential information. A considerable advance in our ideas concerning the soil solution has already resulted from the application of the freezing-point method to soils, as first described by Bouyoucos and McCool (*4*). However, the study of the soil solution in its relation to plant growth is so fundamental that it should be approached from every possible angle, with the hope that eventually we may possess an adequate understanding of the nature of the nutrient medium in the soil and of the modifications produced in this medium by various treatments.

Since the soil solution is constantly undergoing modification, the investigator is often required to make numerous determinations at frequent

[1] Reference is made by number (italic) to "Literature cited," p. 394–395.

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intervals during the growing season at least. This introduces certain practical difficulties in the application of presssure methods. The freezing-point method is most rapid and convenient as a means of studying the approximate total concentrations , but it can not give any information concerning the individual solutes. The method of water extraction has been used rather frequently in past investigations with the intent to determine the amounts of plant foods available to the plant. One of the writers (*9*) has carried out an extensive investigation in which

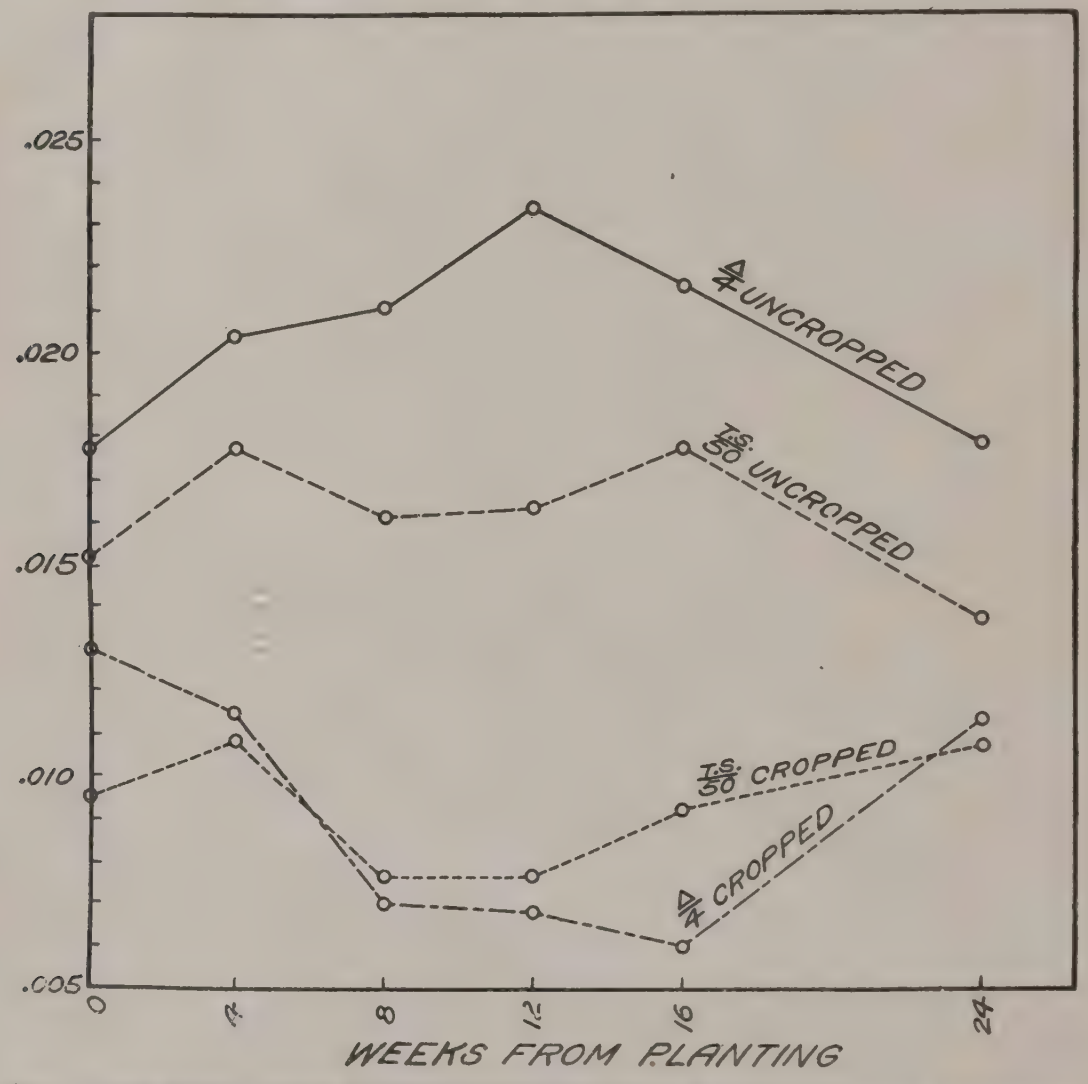


FIG. 1.—Graph showing relation of freezing-point depressions in soil (calculated to 22 per cent moisture) to total solids extracted by 5 parts of water to 1 of soil. Individual data from six soils composited.

the very significant effect of season and crop growth on water extracts of soils was made clear. At the same time the freezing-point method of Bouyoucos and McCool was applied to the soils under investigation, and a general agreement was noted between the values obtained by this method and by the water-extraction method (*6*). Thus the effect of the crop in diminishing the concentration of the soil solution was definitely shown by both methods. At the present time the study of water extracts offers such promise that it has seemed highly important to attempt

to throw further light on the relation between the soil extract and the soil solution. The value of the determination made by the water-extraction method rests primarily on the assumption that a logical relationship exists between water extracts and the soil solution.

In the articles referred to above considerable data were presented to show that in general the larger fluctuations in the total solids found in 1 to 5 water extracts occurred coincidently with similar fluctuations in the freezing-point depressions of the moist soil. Later much more ex-

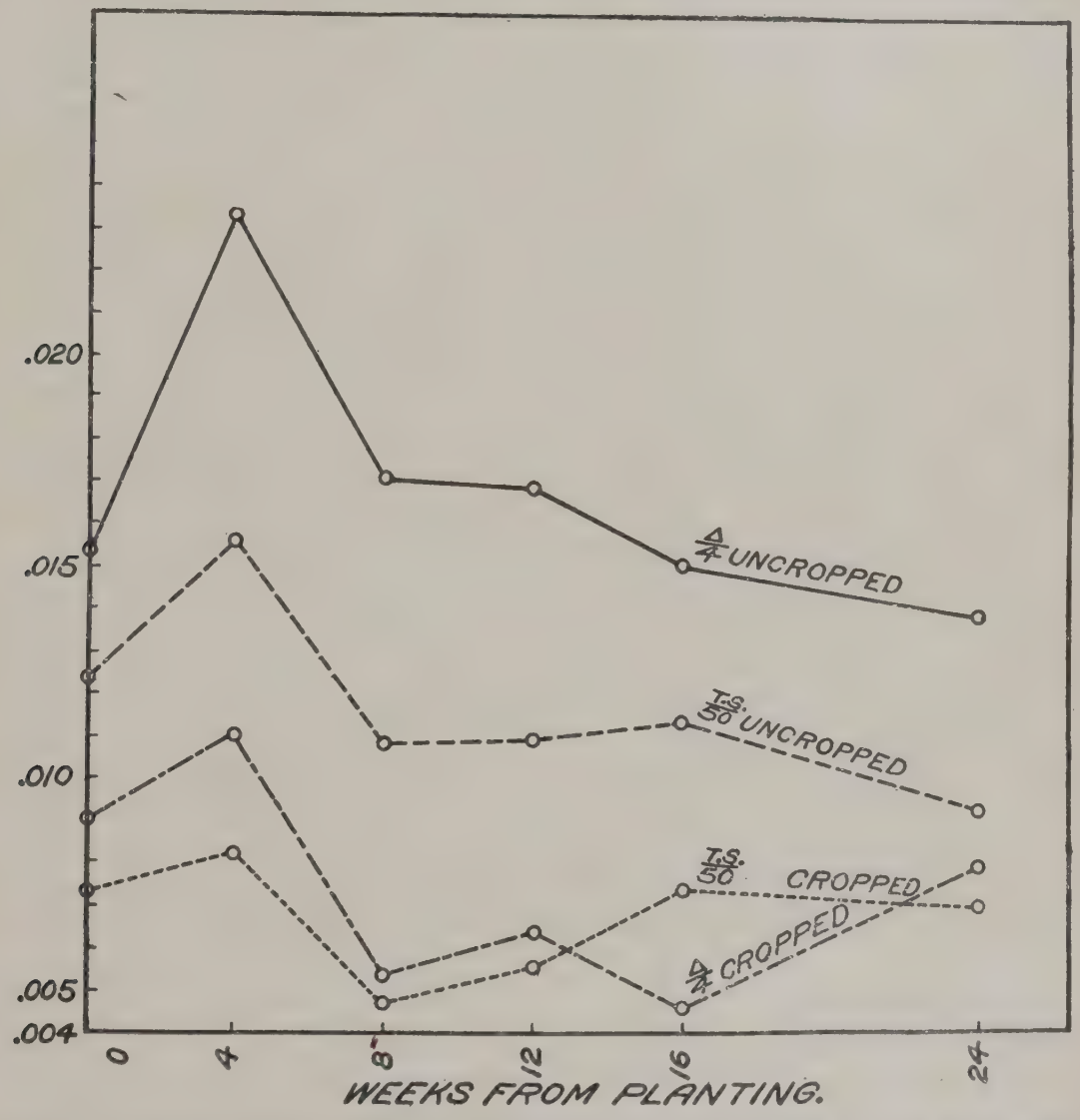


FIG. 2.—Graph showing relation of freezing-point depressions in soil (calculated to 17 per cent moisture) to total solids extracted by 5 parts of water to 1 of soil. Individual data from seven soils composited.

tensive observations were made on this important question, and the data have been plotted in two graphs (figs. 1, 2), one for the group of silty clay loam soils and the other for the various fine sandy loams, the soils being the same as those described in a previous article by Stewart (9). The data for the individual soils have been composited for the present paper. In these graphs the determinations of total solids and freezing-point depressions are plotted for various time intervals, for the soils in both the cropped and uncropped condition. The correlation between

the curves for total solids and freezing-point depressions is on the whole excellent, considering the technical difficulties involved. Chief among these is the uncertainty concerning the free and unfree water in the soil, which, as Buoyoucos (*1*) has clearly shown, markedly affects the concentration of the soil solution. While all the values have been calculated to the same moisture basis, it is not to be expected that this can be done with a high degree of accuracy, since the percentage of unfree water may vary with different moisture contents and perhaps with different concentrations of the soil solution. In both groups of soils there is a somewhat marked divergence between the curves for total solids and freezing-point depressions at a period beginning about 10 weeks after planting the crop. This can reasonably be explained on the basis of certain observations reported in former articles (*6*, *9*). It was shown in these that a larger quantity of very slightly soluble material was dissolved from a soil by a given proportion of water when the soil solution had reached a low concentration as a result of absorption of solutes by the plant. At a certain period, therefore, the cropped soil will yield a higher percentage of dissolved material (not part of the actual soil solution) as compared with earlier periods. This means that the extractions of the cropped and uncropped soils are not on exactly the same basis at all times, and it might be predicted that at the period of low concentration in the cropped soil the proportion of dissolved substances would increase. The inference is substantiated by the experimental data. This generally neglected phenomenon of the effect of the solutes already present in the soil solution in depressing the solubility of substances dissolved from the soil mass by water is thought to be of considerable importance in all studies on soil equilibria by means of water extracts. Finally, it should be emphasized that at no time is there any indication that conclusions based on the water extracts would lead to an erroneous estimate of the general relation between the soil solutions of cropped and uncropped soils. As the authors have pointed out before, the actual differencse would tend to be of greater magnitudes than those calculated from the results on water extracts.

When a 1 to 5 extract of soil is made with distilled water, the quantity of total solids is from 1.5 to 5 times that present in the soil solution, as calculated by the freezing-point method. By the latter method we can calculate the total concentration in the soil solution; but this does not enable us to determine whether or not the relation between the elements in the soil solution is at all similar to that in the soil extracts. Another type of experiment is necessary to give evidence on this point. It was suggested that such evidence might possibly be obtained by determining the concentration and composition of a solution which would remain unchanged when in contact with the soil mass. In other words, if one passed through a sample of moist soil a solution having the

same concentration and composition as the soil solution already present, then it may be assumed that the resultant extract would have the same composition and concentration as the original solution. On the other hand, if the solution used were of different concentration or composition a readjustment of the equilibrium should take place so as to produce a different extract. It was decided to attempt an experiment based on this hypothesis.

Obviously, the preparation of a solution having the same composition and concentration as the soil solution is a matter of great difficulty. The only feasible scheme seemed to be the use of a soil extract concentrated to a point where it would have the same concentration as the soil solution, this concentration being determined by the method of Bouyoucos and McCool. It was reasonable to assume that in such a solution there would exist, between some of the most important elements, a relation very similar to that found in the actual soil solution—that is, the solution of the free water with the soil at approximately optimum moisture content. In order to limit as far as possible the quantity of solutes dissolved from the soil mass, an extract was made with cold water, and only ½ part of water was used to 1 part of soil. The time of contact was limited to that necessary for complete admixture. Filtration was made through a Buchner funnel, and final clarification was effected with the use of a Pasteur filter. A separate portion of the soil was then made up to its optimum water content, and the freezing-point depression was carefully determined. The extract of the soil made in the manner described was then concentrated on a hot plate, meanwhile passing through the solution a stream of carbon-dioxid gas in order to prevent any precipitation. Finally the volume of the concentrated extract was adjusted with distilled water so that it had exactly the same freezing-point depression as that of the moist soil. This solution was used in extracting the moist soil (1 part of soil to ½ part solution). Careful analyses were made of the extract before and after contact with the soil, and the results were compared.

Before the data are considered it should be recalled that ordinarily in a water extraction from 2 to 5 times as much total solids are dissolved as are actually present in the soil solution, and this is true with the extractions now considered. Under certain conditions, however, it is possible to obtain an extract which contains a comparatively small quantity of dissolved substances in addition to that originally present in the soil solution, as indicated by the method of Bouyoucos and McCool. For example, a sample of soil 9, having a freezing-point depression of 0.148° C. at 17 per cent moisture gave in a 1 to ½ extract only about 1.16 times the quantity of total dissolved solids equivalent to this depression. In this case the unfree water was determined directly by dilatometer measurements (*1*). Such a result apparently can be obtained only with

a soil having a low percentage of colloidal material and having a fairly high concentration in its soil solution, which exercises a repressive effect on the solubility of certain soil constituents as previously explained.

In Table I the results of the equilibrium studies with three different soils are presented. Comparisons are made between the composition of the concentrated extracts and the same extracts after treatment with the soil. It will be noted that the total concentration has suffered practically no change, as shown by the freezing-point depressions, conductivity determinations, and proportion of total solids. Also, the concentrations of potassium, magnesium, calcium, nitrate, and sulphate agree within the limits of experimental error. The agreement for sodium is less perfect, but considering the small quantities involved the differences are also probably within the limits of error. In one case more phosphate is found in the re-extract, and in two cases the agreement is fairly close. In one case the two silica determinations agree almost perfectly, and in two cases silica seems to have been retained by the soil. It is very difficult to explain the action of this radicle, first because of the chance of contamination of the solution from glass vessels and secondly because of the numerous types of silicates possible with varying proportions of silica.

While the agreement between the extracts and re-extracts is on the whole remarkably close, it might be objected that the conditions for the attainment of equilbrium were inadequate and that another extract having a different composition might also remain unchanged by the soil. In order to test this possibility extracts were made of soils 9 and 15 in the previously described manner, and then potassium sulphate was added to the extracts so as to double approximately the concentration of potassium. These modified extracts were then concentrated until they had the same osmotic value as the soil solutions, and re-extracts were made as in the first experiment. The composition of the different solutions is given in Table II. It is evident that in this experiment the soil has had a marked effect on the extract. There is very much less potassium in the re-extract than in the original extract, but the decrease of potassium is accompanied by an increase in the quantity of calcium and in one case of sodium. In one case there is a slight decrease of sulphate. The other elements are not greatly changed, nor is the total concentration very different in the two cases. It seems clear that a rearrangement of the solutes has taken place in this case which did not occur in the first experiment. In other words, the extract introduced was different in composition from the soil solution already present, with the result that certain chemical reactions took place forming an entirely new soil mass—soil solution system.

TABLE I.—*Extraction of soil with its concentrated extract*

Soil No.	Description of extracts.	Freezing-point depressions.	Specific resistance.	Composition of extracts.								
				Total solids.	Potassium (K).	Calcium (Ca).	Magnesium (Mg).	Sodium (Na).	Nitrate (NO_3).	Phosphate (PO_4).	Sulphate (SO_4).	Silica (SiO_2).
		°C.	*Ohms.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1C	Concentrated extract	0.064	520	1,944	50	148	74	36	342	3	182	61
	Same after passing through soil	.063	550	1,904	43	148	74	31	348	4	183	33
9	Concentrated extract	.041	610	1,596	46	228	27	41	366	3	116	52
	Same after passing through soil	.043	590	1,688	41	264	27	43	384	4	108	25
15	Concentrated extract	.066	250	2,640	84	308	61	48	558	6	168	26
	Same after passing through soil	.064	260	2,680	72	320	58	34	552	6	170	25

TABLE II.—*Extraction of soil with concentrated extract containing added potassium sulphate (K_2SO_4)*

Soil No.	Description of extracts.	Composition of extracts.								
		Total solids.	Potassium (K).	Calcium (Ca).	Magnesium (Mg).	Sodium (Na).	Nitrate (NO_3).	Phosphate (PO^4).	Sulphate (SO^4).	Silica (SiO_2).
		P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
9	Concentrated extract plus potassium sulphate	1,652	76	212	33	122	312	3	166	40
	Same after passing through soil	1,896	52	280	31	129	333	4	161	40
	Increase or decrease in concentration	+244	− 24	+ 68	− 2	+ 7	+21	+1	− 5	.....
15	Concentrated extract plus potassium sulphate	2,408	277	236	41	72	.....	4	413	24
	Same after passing through soil	2,592	119	300	41	109	.....	3	369	22
	Increase or decrease in concentration	+184	−158	+ 64	.....	+ 37	.....	−1	− 44	− 2

All the experiments just discussed would seem to justify the conclusion that in a concentrated extract the relation between the various elements may be very similar to that existing in the soil solution. In seeking an explanation of the results it is first essential to describe the components which probably enter into a water extract of a soil. In the first place, these would include the constituents of the soil solution diluted by the added water. This diluted soil solution would then tend to bring into solution constituents which were not present in the soil solution itself. Thus, in no case would the solvent be pure water but rather a solution the composition and concentration of which would vary with the soil solution. It is plausible to assume that the solvent thus formed would bring into solution principally either "adsorbed" salts or easily soluble chemical compounds, originally derived from the more resistant minerals. Finally, a certain quota of this very slightly soluble material would come into solution, and the total quantity dissolved would depend at least in part on the total volume of water as well as on time and temperature. This latter fraction of the soil extract would ordinarily form only a small portion of the total dissolved material. Evidence for this view has been presented previously (*6*, *9*) and is also upheld by certain experiments of Bouyoucos with regard to the solubility of soil minerals (*2*). It would follow, therefore, that if the adsorbed or immediately soluble material has the same relative composition as that already present in the soil solution, then the water extract might also retain similar relations. It is impossible at present to obtain direct evidence to this effect, but an experiment was carried out from which certain inferences may be drawn. A large quantity of moist soil (silty clay loam 1) was placed in a Buchner funnel and leached with the least possible proportion of distilled water, about ⅛ part of water to 1 part of soil. Two subsequent leachings were made with similar proportions of water. These three extracts were then analyzed for the most important elements, and the ratios

between them were calculated by dividing the concentration of each element by the sum of the concentrations of all the elements determined (Table III). The ratios were found to be very similar in the three extracts, the agreement for several elements being especially close in the first two extracts. In the first extract the larger proportion of solutes present were probably derived from the soil solution, while the subsequent extracts represented to a greater degree previously undissolved fractions of the soil. The results would, therefore, seem to indicate that in concentrated extracts there is a great similarity in composition between the soil solution and the extract containing the substances which immediately go into solution on the addition of a slight excess of water. Even with nitrate, which might be supposed to have such a high degree of solubility that the total quantity present would be contained in the soil solution, it is probable that a certain proportion is held in some adsorbed or undissolved form. If an extract of the soil be made, a readjustment takes place because of the great dilution of the soil solution, and the total quantity of adsorbed nitrate would be greatly diminished, even though the partition ratio between solution and soil remained constant. Thus, it is possible to extract nearly all the nitrate present but difficult or impossible to remove the last traces.

TABLE III.—*Composition of successive leachings of soil*

[8 parts soil to 1 part water.]

Solute.	First leaching.		Second leaching.		Third leaching.	
	Concentration of solution.	Ratio of individual solutes to total.	Concentration of solution.	Ratio of individual solutes to total.	Concentration of solution.	Ratio of individual solutes to total.
	P. p. m.	*Per cent.*	*P. p. m.*	*Per cent.*	*P. p. m.*	*Per cent.*
Nitrate (NO_3)	425	57.0	195	53.0	133	46.0
Calcium (Ca)	90	12.1	45	12.3	46	15.8
Magnesium (Mg)	88	11.8	43	11.8	34	11.6
Potassium (K)	22	3.0	15	4.1	13	4.4
Sulphate (SO_4)	121	16.2	67	18.4	66	22.6

With regard to phosphate the case is not so clear. Most of the extraction studies described in previous articles have indicated that the various extracts are saturated with respect to phosphate. Thus, if the extract were concentrated without precipitation the concentration of phosphate should be considerably greater than in the soil solution. Since, however, the adsorption or precipitation of phosphate by the soil is a relatively slow process, in the present experiment the time may have been insufficient for readjustment of the equilibrium. From our previous experiments we should be inclined to infer that the concentration of phosphate in the soil solution is usually very low, but that immediate replacement occurs as phosphate is absorbed by the plant, thus producing a constant concentration of phosphate over long periods of time.

That there exists some sort of definite and reversible state of equilibrium between the soil mass and the soil solution for any given set of conditions is suggested by another experiment. Two soils were treated with water in the proportion of 1 part of dried soil to 1 part of water. After the soil and water were thoroughly mixed the resultant mixtures were allowed to dry at room temperature until they reached the optimum moisture content. Freezing-point depressions were then made and compared with determinations made on samples of the same soils simply moistened to optimum water content. The data given below show that the agreement is, at least in these two cases, almost perfect.

TABLE IV.—*Freezing-point depressions of soil at optimum moisture content and of treated soil evaporated to optimum moisture content*

Description of soil.	Freezing-point depressions.
	°C.
Soil 1C at optimum moisture content	0.063
Soil 1C after mixing 1 to 1 with water and allowing to evaporate to optimum moisture content	.062
Soil 9 at optimum moisture content	.045
Soil 9 after mixing 1 to 1 with water and allowing to evaporate to optimum moisture content	.047

In other words, although several times as much material was brought into solution as was contained in the soil solution at optimum water content when the excess water was added, these dissolved substances were immediately removed from solution on lowering the moisture content. This, of course, does not mean that the concentration of the soil solution may not easily be altered by the addition of soluble salts, as will be discussed presently.

If the general method of studying soils by means of their water extracts is of value, then it becomes of considerable importance to determine the most suitable conditions for making the extract. The technic might be based on either one or two general objectives, first the attainment of equilibrium (as nearly as possible final) for a given proportion of water, and, second, the limitation of the extract as far as was practicable to the material actually existing in the soil solution. In the first case a long period of contact and continuous shaking would be essential; in the second case the time would be limited to that necessary for complete admixture of soil solution and added water. In order to determine the magnitudes of dissolved substances under varying conditions, extracts of 3 soils were made by various methods as follows: (*a*) 1 part soil to 5 parts water, as described by the Bureau of Soils of the United States Department of Agriculture; (*b*) 1 part soil to 5 parts water, shaking for 1 week; (*c*) 1 part soil to 1 part water, as in (*a*); (*d*) 1 part of soil to 1 part water, shaking for 1 week.

In Table IV the results on extracts obtained by these different methods are presented, all calculated to parts per million of the dry soil, so that comparisons may be made on the same basis.

If the total solids are considered, it will be noted that the magnitudes are very similar except in the case of the 1 to 5 extract shaken for 1 week. More potassium is extracted by a 1 to 5 extract than by a 1 to 1 extract, but the quantities are essentially the same whether the time is 40 minutes or 1 week. The calcium, magnesium, and sulphate may be appreciably increased during the week's contact when the proportion is 1 to 5 but not when the proportion is 1 to 1. Nitrate is not greatly changed in a 1 to 5 extract by the increased time of extraction. In the 1 to 1 extract in one case of a heavy-textured soil there is a decrease after 1 week, and in another case of a light-textured soil there is an increase. Very probably biological action is concerned in these changes. Phosphate is increased markedly in the 1 to 5 extract as compared with the 1 to 1 extract.

Several fairly definite deductions may be drawn from the data just presented. When a smaller proportion of water to soil is used, as 1 to 1, there is only slight increase in dissolved substances with the period of 1 week as compared with a shorter period, although some changes in nitrate may result from biological action. There would not seem, therefore, to be any advantage in the longer period of contact; in fact the biological changes would make such a procedure undesirable. In the 1 to 5 extracts there is a significantly increased solution of various elements (particularly calcium and magnesium) during the period of a week. This must be due to the solution of soil minerals, more of which are dissolved in the 1 to 5 extract because of the greater dilution of the solvent, as previously explained. Phosphate is in a somewhat different category from the other elements in that the total quantity dissolved is somewhat directly dependent upon the volume of the solvent. As was stated before, to a certain extent the solution is always saturated with respect to phosphate.

TABLE V.—*Comparison of extracts of soil prepared by various methods*

Soil No.	Time of extraction.	Ratio of soil to water.	Composition of extracts calculated to basis of water-free soil.						
			Total Solids.	Potassium (K).	Calcium (Ca).	Magnesium (Mg).	Nitrate (NO_3).	Phosphate (PO_4).	Sulphate (SO_4).
			P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1C	40 minutes	1 : 1	680	24	62	26	80	1	60
	1 week	1 : 1	612	26	47	24	54	1	79
	40 minutes	1 : 5	636	38	47	25	128	6	.....
	1 week	1 : 5	1,034	37	74	33	126	5	81
9	40 minutes	1 : 1	510	16	68	7	68	2	27
	1 week	1 : 1	562	20	69	11	114	2	37
	40 minutes	1 : 5	503	33	70	10	114	7	29
	1 week	1 : 5	806	38	100	19	128	8	30
15	40 minutes	1 : 1	532	23	59	13	78	2	41
	1 week	1 : 1	524	30	51	16	63	1	45
	40 minutes	1 : 5	582	31	52	15	116	7	.....
	1 week	1 : 5	836	38	91	24	145	10	55

The application of the foregoing conclusions would seem to indicate that soil extracts should be made with a small proportion of water and for a short period. It would probably be desirable to use not more than 1 part of water to 1 part of soil, but in many cases this may be impracticable, so that 1 to 5 extracts must suffice. It is true that special studies of soil equilibria must take into account long-continued solvent action, but in attempts to gain some idea of periodic changes in the soil solution the technic should be directed toward lessening the solution of material not actually present in the soil solution. This aim is less possible of attainment in proportion as the volume of water or time of contact with the soil is increased. It is not evident that attempts to reach approximate final equilibrium by large excess of water or long shaking are likely to result in more accurate knowledge of the soil solution as it exists at any given moment. On the contrary, the increase in solutes is derived from substances not actually present in the soil solution, and their solubility is in part conditioned on the concentration of the soil solution, the variable under investigation.

In concluding this discussion it may be well to summarize briefly our present point of view with regard to the soil solution based on recent researches in this and other laboratories. All the evidence supports the general views expressed by Cameron (5) a number of years ago to the effect that soil phenomena must be considered as dynamic. His criticisms of the older methods of study by means of hydrochloric-acid extracts of soils, analyses of total quantities present in the soil, etc., are found to be entirely justified. It is now generally recognized, however, that Cameron's conclusions with regard to the nature of the soil solution were not sufficiently far-reaching. It is certain that the soil solution is not simply a solution saturated with respect to all the original mineral components of the soil and tending to approach a constant composition. The original soil minerals themselves doubtless have a very slight solubility in pure water, but the soil solution of a normally occurring soil is something quite different. The solvent is never pure water, but rather a solution of salts and organic matter, accompanied by carbon dioxid, oxygen, and other gases. The soil solution at any given moment is the resultant of the cumulative effect of the continuously varying solvent on the soil minerals. The actual concentration of the solution is governed principally by the equilibria existing between the dissolved substances and the immediately soluble or absorbed substances. It is possible that these latter may be removed almost completely from the soil mass by an excess of water. The soil solution in contact with the residual soil has a very low concentration, and this is not readily increased by the solvent action of pure water. To a lesser degree a similar state of affairs results when the dissolved or immediately soluble components of the soil are removed by a crop. This effect may be of long duration, or, on the other hand, the concentration of the soil extract with respect to many solutes

may easily be increased by the addition of soluble salts. Bouyoucos and Laudeman (*3*) have shown, moreover, that this increase of concentration occurs immediately and in most cases is not altered over a long period of time.

Theoretically, also, it is very apparent that the soil solution or extract may be increased in its concentration of a given element by the addition of a soluble salt. A simple case will illustrate this fact. A saturated solution of slightly soluble silicates of potassium, for example, can be prepared by shaking the finely divided minerals with water. The concentration of potassium in the solution is limited by the solubility of the components of this particular system. However, the addition of another component of different solubility, such as potassium chlorid, will increase the concentration of potassium in the solution, although the solubility of the potassium silicate may possibly be diminished because of the increased concentration of the potassium ion. In the same way the soil solution is saturated only with respect to the particular system existing at any given moment. In general it is not saturated with respect to any particular ion, so from theoretical considerations there is no reason to accept the earlier statements of Cameron that the chemical equilibria would require the precipitation of added salts with a tendency to maintain a constant composition in the soil solution. The fact that water extracts of soils become more dilute with each increase in the proportion of water used gives evidence to show that the solubility of the original soil minerals is not the chief factor governing the concentration of the soil solution.

Presumably in the actual soil solution the increase of concentration due to the addition of soluble salts will in part be limited by the removal from the dissolved to the absorbed phase. When an excess of water is employed, however, as in making an extract, nearly all of the added solutes will appear in solution or be represented by equivalent quantities of other substances, as is shown, for example, in the well-known exchange of bases. The total quantity of absorbed substances would be a function of the concentration of the surrounding solution, which would vary with the moisture content of the soil or volume of water used in making an extract. In extraction procedures there would occur, of course, a very great dilution of the soil solution. While the latter would be increased in concentration by the addition of soluble salts, the evidence at hand does not indicate that all the added salt would necessarily be effective in increasing the concentration of this soil solution even when the water extracts contained the total or equivalent quantities of the elements added. It is reasonable to assume, however, that the "adsorbed" substances are capable of easily replenishing the soil solution when its concentration is decreased as a result of withdrawals by the plant, new soil solution-adsorption systems being formed continuously during the season.

SUMMARY

(1) Seasonal studies on cropped and uncropped soils have shown that water extracts reflect the principal fluctuations taking place in the soil solution as indicated by the freezing-point method.

(2) A soil extract is composed chiefly of the solutes present in the soil solution plus substances dissolved from "adsorbed" or easily soluble components of the soil. This latter fraction of the soil extract is dependent in part on the concentration and composition of the soil solution, since the solutes of the latter exert a depressing effect on the solubility of certain soil constituents. This fact is believed to be of great importance in studies of chemical equilibria in soils.

(3) A new method is suggested for indicating the relations between the chemical elements in the soil solution. Extracts were prepared which did not change appreciably in composition or concentration on contact with the soil. The consideration of the equilibria involved suggests the probability that the ratios between most of the important elements are very similar in concentrated soil extracts and in the soil solution. It is concluded that analyses of suitable soil extracts and determinations of freezing-point depressions may frequently permit a calculation of the concentration and approximate composition of the soil solution.

(4) Various methods of making water extracts have been compared. The data obtained suggest that in seasonal studies extracts should be made with the smallest proportion of water to soil practicable and with the time of contact limited to that necessary for thorough admixture. In routine work 1 to 1 or 1 to 5 extracts are convenient and satisfactory.

(5) Further experimentation has confirmed previous conclusions that the soil solution fluctuates in composition and concentration with every environmental change and with crop growth.

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EFFECT OF SEASON AND CROP GROWTH ON THE PHYSICAL STATE OF THE SOIL

By D. R. HOAGLAND and J. C. MARTIN, *Division of Agricultural Chemistry, California Agricultural Experiment Station*

Investigations previously reported by this laboratory [1] have shown definitely that the soil solution is extremely variable in its composition and concentration, as indicated by water extracts or by the freezing-point method of Bouyoucos and McCool.[2] Recently McCool and Millar[3] in an extensive series of field studies have upheld this conclusion. In all these investigations it has been demonstrated that the absorption of solutes by the plant may have a very pronounced influence on the soil solution at certain periods and may bring about a very striking decrease in the concentration of nitrates and other constituents. Moreover, this condition may persist for a long time. During the course of our experiments it was noted that the state of dispersion of the colloidal matter in the various soils fluctuated in a most decided manner under the influence of the different treatments. It was decided, therefore, to make a systematic series of observations relating to this point.

The soils used were kept under controlled conditions in tanks as described by Stewart.[4] Both cropped and uncropped soils were compared under otherwise identical conditions. The principal measurements were made on a number of tanks of silty clay loam soil, clay in which various crops were grown—namely, corn, barley, potatoes, beans, and beets. There were three tanks of barley, containing, respectively, 24, 50, and 71 plants. All soils were kept at approximately optimum moisture content by the addition of distilled water. At frequent intervals during the growth of the crops samples of soil were taken for examination.

In order to study conveniently the changes in the water-soluble constituents, conductivity measurements were made on water extracts of the soil. These were made by thoroughly mixing 1 part of moist soil with 2 parts of distilled water and filtering through filter paper. This

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[3] MCCOOL, M. M., and MILLAR, C. E. OP. CIT.

[4] STEWART, Guy R. OP. CIT.

method gives results of the same relative values as those obtained by determining the total solids in water extracts or by estimates based on depressions of the freezing point in the soil itself. It is justifiable to assume that the conductivity measurements give at least a rough idea of the changes taking place in the soil solution under the various conditions.

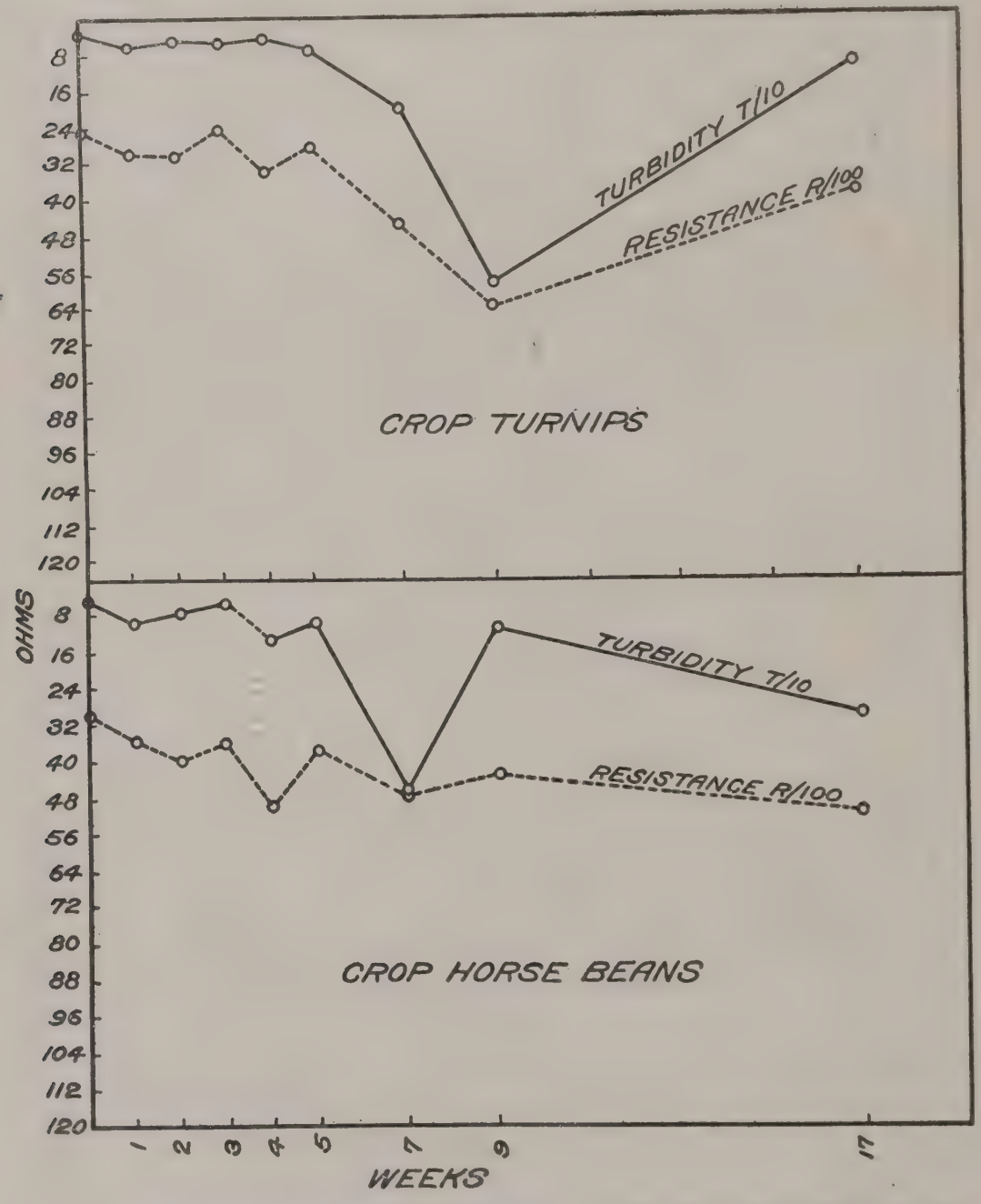


FIG. 1.—Effect of crop on physical state and electrolyte concentration of the water extract of the soil.

Unfortunately no methods exist which permit the determination of the exact kinds or quantities of colloidal matter in the soil. We can measure only approximately certain resultant effects by the use of empirical procedures. Of these, turbidity observations are doubtless as valuable as any others. In the present experiments the samples of soil were mixed with water, in the proportion of 1 part of soil to 2 parts of water, and the

soil suspensions were poured into burettes. After 24 hours the upper 10 cc. were carefully pipetted off into weighed dishes, and the total residue was estimated after evaporation and drying at 100° C. While such a method unquestionably leaves much to be desired, it is nevertheless apparent that considerable changes in the colloidal state of the finer

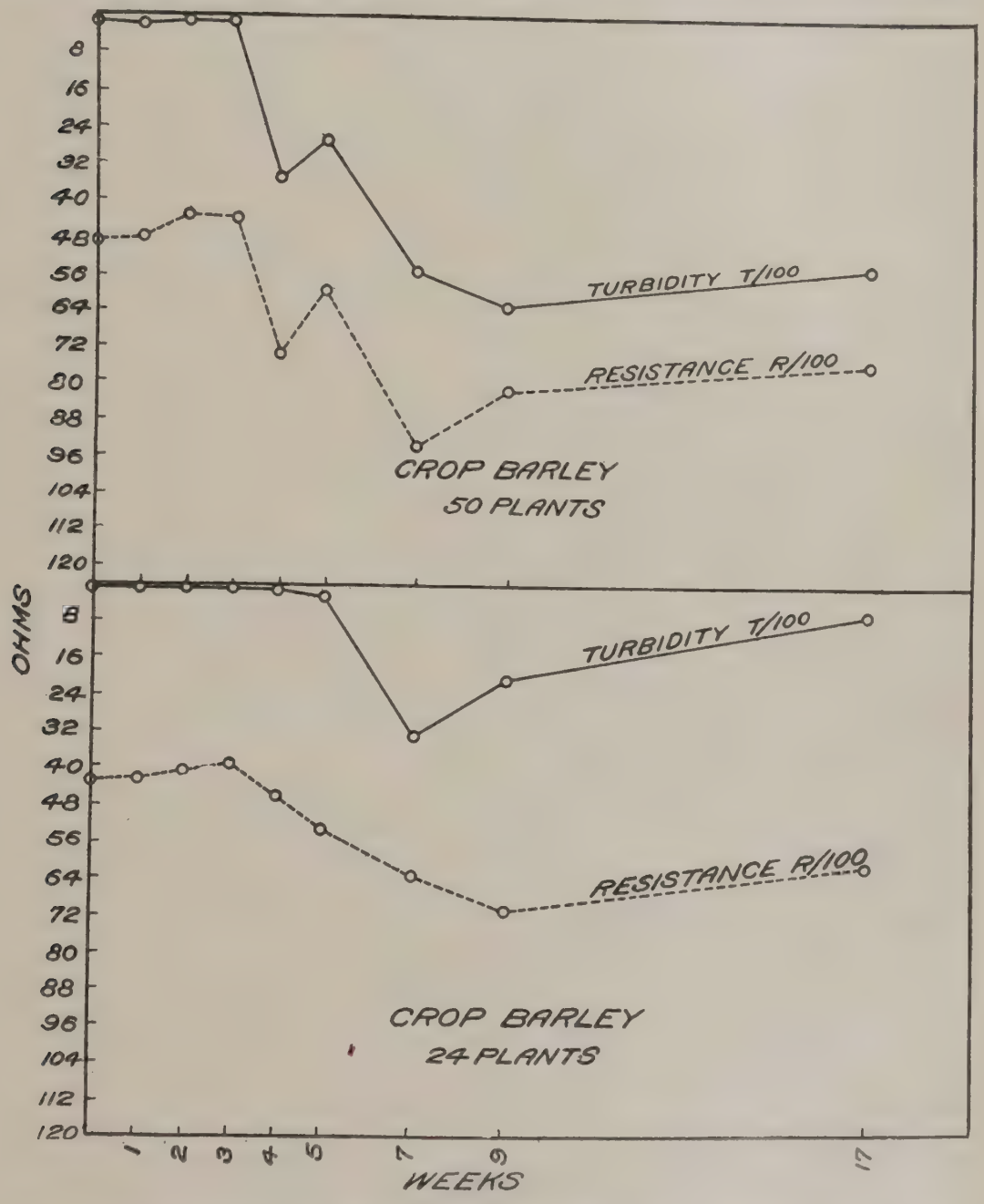


FIG. 2.—Effect of crop on physical state and electrolyte concentration of the water extract of the soil.

soil particles are reflected in the quantities of suspended material obtained in this manner.

The data have been expressed in the form of graphs with the time (in weeks) plotted against values expressing the magnitudes of the suspended material and also against the resistances of the extracts in ohms. Since the concentration of the solution varies inversely as the resistance, the scale has been inverted to bring out the relations more clearly. (Fig. 1–4.)

It is evident that there exists a very good general correlation between the quantity of soluble constituents in the soil and the quantity of suspended material and that in both cases the magnitudes undergo very marked variations coincidentally with seasonal changes and crop growth. These fluctuations are far more pronounced, however, in the cropped

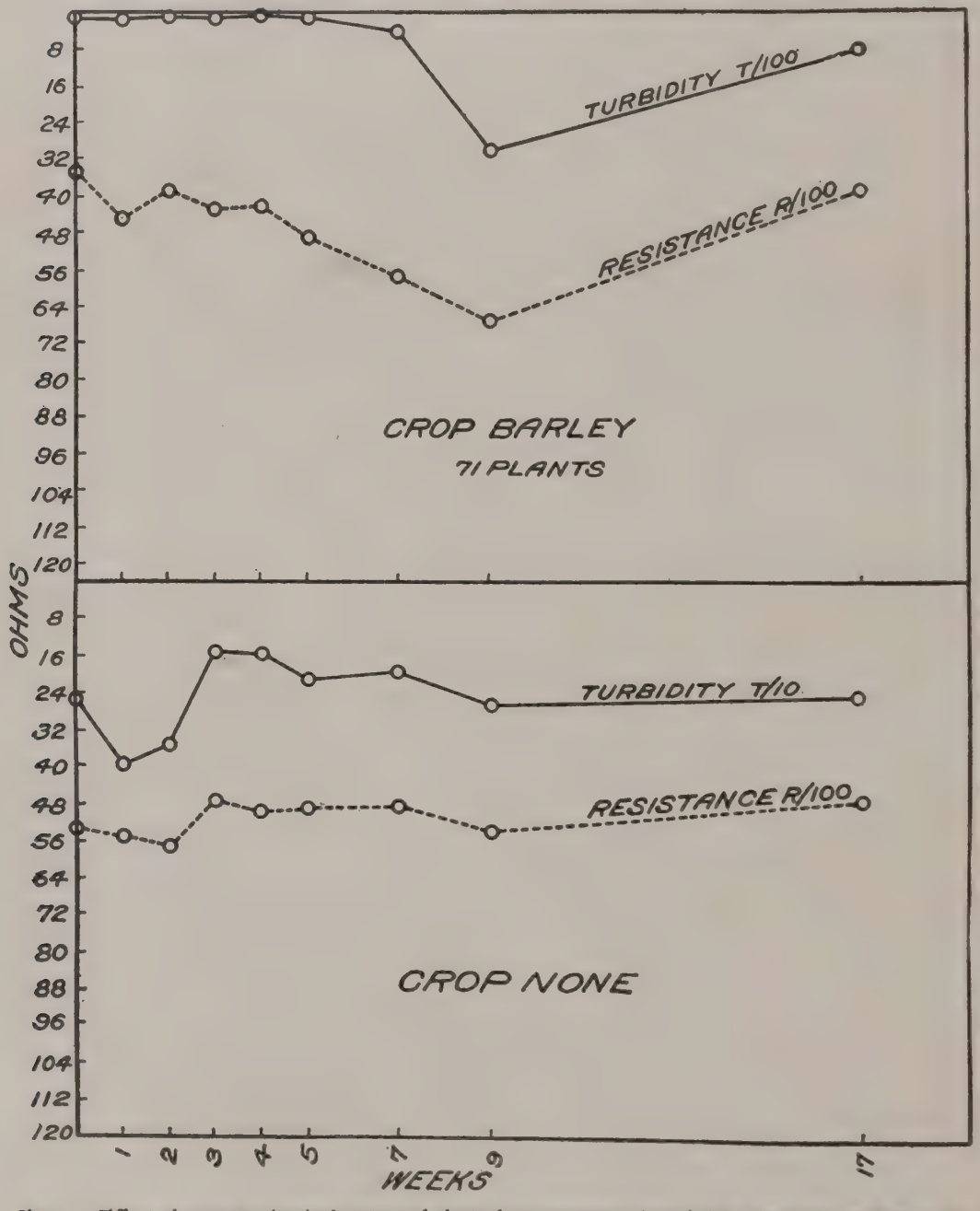


FIG. 3.—Effect of crop on physical state and electrolyte concentration of the water extract of the soil.

soils than in the uncropped soils. In other words, it is a fair conclusion that the absorption of solutes by the plant has lowered the concentration of the soil solution at a period of 8 or 10 weeks after planting and that the physical state of the soil has undergone an equally definite change. It can scarcely be doubted that there is some definite relation between the concentration and composition of the soil solution and the

physical state of the soil. That this correlation is only approximate is not difficult to explain, even if we assume that the factors mentioned above are the only ones to be considered. The quantity of suspended material obviously can not bear an exact relation to the concentration of the solution throughout all ranges. At a certain point the supernatant liquid will become almost clear, and while further increases in the con

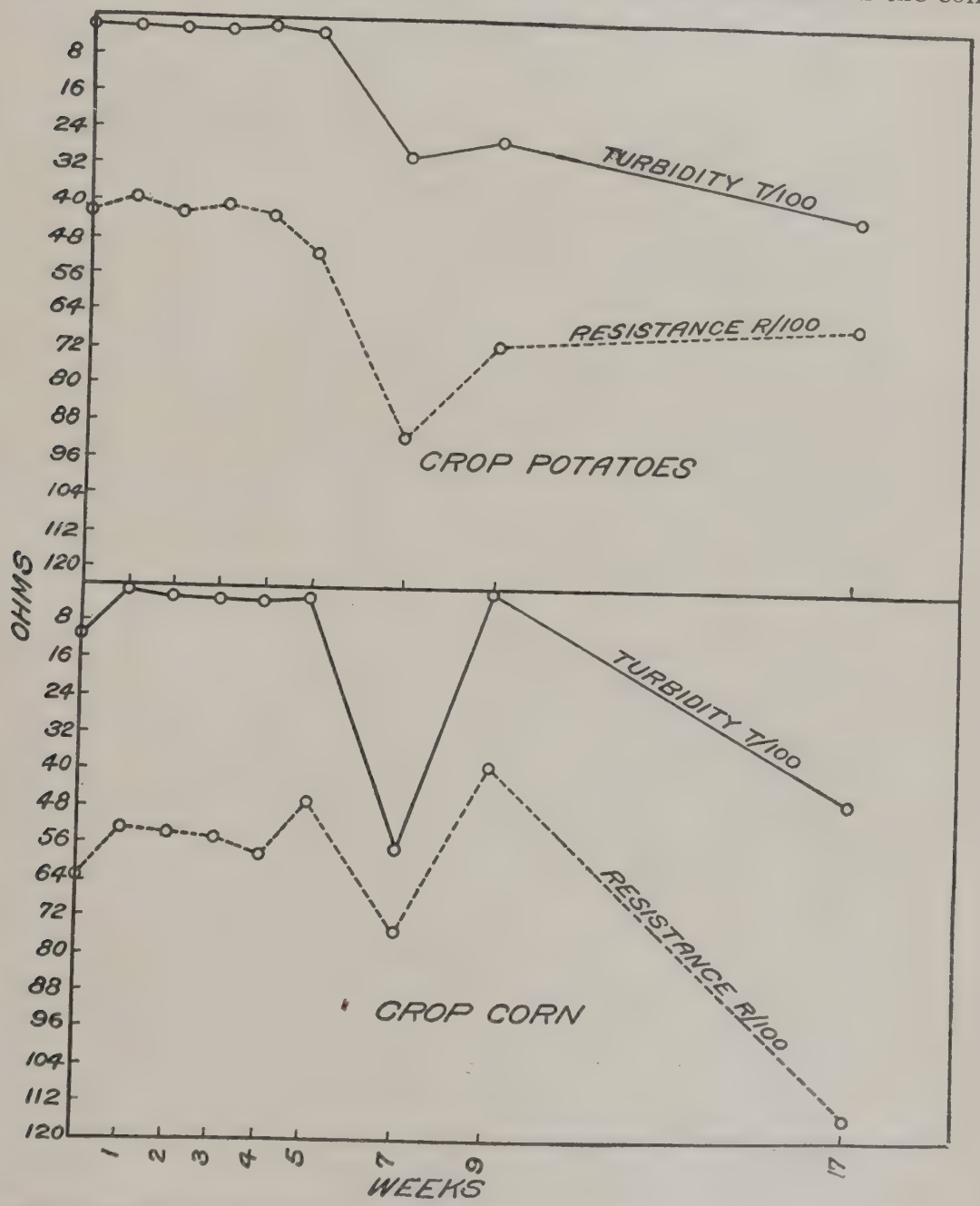


FIG. 4.—Effect of crop on physical state and electrolyte concentration of the water extract of the soil.

centration of electrolytes will diminish the resistance almost proportionally, no further change can be shown in the physical state of the soil as measured by the present method. Moreover, the conductivity is a resultant measurement expressive of the concentration or mobility of all the ions present. It is not true, however, that equivalent quantities of different ions have equal effects on the colloidal state of the soil.

The effect of various salts on the flocculation of soils has been studied in many investigations. Sharp [1] in an extensive series of observations has presented evidence to show that a remarkable change is produced in the physical state of the soil by the addition of various salts and subsequent washing of the soil with water. This change in the degree of dispersion of the colloids is attributed to the formation of new silicate compounds which give to the soil its new properties.

These investigations by Sharp have all dealt with rather extreme salt effects, such, for example, as might occur in "alkali" soils or heavily fertilized soils. So far as known, no study has been made of the changes which may take place in soils because of the normal fluctuations in the soil solution under varying conditions of cropping and season. In such cases the total quantity of salts dissolved in the soil solution is extremely small, and it might be questioned whether these could have any appreciable effect on the physical state of the soil. However, an analysis of the data presented by Stewart [2] and Hoagland [3] brings out the fact that relatively enormous fluctuations may take place in the soil solution. The growth of a crop, for example, in certain instances may reduce the concentration of the soil solution to an extremely low point. Recently McCool and Millar [4] have presented extensive data to show that in the field very profound changes may occur in the soil solution as a result of cropping, moisture variations, biological activities, rainfall, etc. Apparently all of these fluctuations in the soil solution may be reflected in the physical state of some at least of the soil constituents.

Since the effect of cropping is to reduce the water-soluble constituents of the soil and the concentration of the soil solution, it might be predicted on the basis of the foregoing discussion that soils which had been cropped would show a physical condition distinctly different from the same soils kept uncropped. In order to decide this point more definite turbidity determinations were made on a number of different soils. Except that one tank of each soil had been cropped for four years and one tank had been kept without crop for three years, the soils were maintained under identical conditions. Originally both portions of the soil were from one sifted, homogeneous mass. The details of treatment have already been described in an article by Stewart.[5] Chemical analyses and conductivity measurements on water extracts, as well as freezing-point depressions on the moist soil, all pointed to the fact that the uncropped soil yielded a soil solution of higher concentration than did the cropped soil. The data contained in Table I give evidence that these differences were reflected in the physical state of the soils. It is particularly easy to demonstrate this relation for the silty soils, but even the sandy soils display the same tendency.

[1] SHARP, L. T. op cit.
[2] STEWART, Guy R. op. cit.
[3] HOAGLAND, D. R. op. cit.
[4] MCCOOL, M. M., and MILLAR, C. E. op. cit.
[5] STEWART, Guy R. op. cit.

TABLE I.—*Relation of physical state to the electrolyte concentration of the soil extract*

Soil No.	Condition of soil.	June 3.		July 26.	
		Turbidity.[a]	Specific resistance.	Turbidity.[a]	Specific resistance.
			Ohms.		*Ohms.*
1	Cropped	170	4,500	.....	.....
	Uncropped	80	2,900	.....	.....
2	Cropped	330	5,000	1,400	6,800
	Uncropped	190	4,200	640	5,100
3	Cropped	230	4,300	6,620	6,900
	Uncropped	160	3,700	220	4,100
4	Cropped	170	4,300	100	3,900
	Uncropped	90	2,900	60	3,000
5	Cropped	290	4,800	410	5,800
	Uncropped	100	2,700	160	4,100
6	Cropped	270	4,500	.....	.....
	Uncropped	180	3,400	.....	.....
7	Cropped	1,120	6,500	.....	.....
	Uncropped	950	5,900	.....	.....
8	Cropped	340	5,800	.....	.....
	Uncropped	300	3,700	.....	.....
9	Cropped	1,090	8,000	.....	.....
	Uncropped	1,070	7,500	.....	.....
10	Cropped	970	7,400	1,060	10,400
	Uncropped	1,330	7,000	610	4,400
11	Cropped	1,730	5,800	2,100	5,900
	Uncropped	130	3,300	160	3,400
12	Cropped	1,220	12,400	1,320	16,900
	Uncropped	1,050	6,500	1,160	8,100
14	Cropped	1,320	8,700	1,260	9,200
	Uncropped	1,140	6,300	1,420	6,500
1C	Cropped	.....	.....	2,010	9,000
	Bin	.....	.....	150	4,300

[a] Expressed in milligrams per 100 cc.

It has already been pointed out that under certain conditions of storage a soil may accumulate a large amount of soluble constituents. It was thought to be of interest to compare a sample of soil which had been kept in a bin for several years in a slightly moist condition with a sample of the same soil cropped for several years. The two samples displayed widely different concentrations of electrolytes, and the turbidity measurements indicate that 20 times as much material was kept in suspension in the cropped soil. These samples demonstrate the extreme effects which may occur, even without fertilization or leaching.

Sharp [1] has shown that salt-treated soils washed with water are made far more impervious than soils washed with water without previous treatment. If, however, a soil is very completely leached with distilled water after stirring, an extremely impervious condition of the soil results. At the same time the final leachings are exceedingly dilute, and the concentration of solution in the leached soil is so small as to be scarcely determinable. In Sharp's experiments the impervious condition of the

[1] SHARP, L. T. op. cit.

soil is considered to be the result of the formation of certain new silicates. Possibly in soils leached with water and not containing an excess of salts the dispersed condition may be the result of the almost complete removal of electrolytes from the films of solution surrounding the soil particles. To a lesser extent the same thing occurs when the soil solution is depleted through absorption of solutes by the plant. None of the data presented in this paper, however, are of such a nature as to permit of any conclusions with regard to these very difficult questions concerning the colloid chemistry of the soil.

Neither is it possible to state definitely the effects of the fluctuating soil solution on the physical state of the soil under field conditions. A sample of soil may be maintained in a relatively pervious state even after long washing, provided the compound particles of soil are not disturbed by stirring or mixing while the soil is saturated with moisture. Nevertheless, it is probable that the soil in the field is subject to certain modifications in its physical state which are merely accentuated when the laboratory tests are carried out.

It is interesting to speculate on the indirect effects of the changes in the physical condition of the soil noted in these experiments. It is entirely possible that such changes may be of considerable importance. The aeration, resistance to root penetration, ease of cultivation, percentage of unfree water, etc., are very probably affected to a greater or less degree, and these alterations in the soil conceivably may have an important influence on the growth of microorganisms or plants. In any case, it is highly desirable to make observations on all the effects, direct and indirect, which may be correlated with the changing concentration or composition of the soil solution. It should be strongly emphasized that in studies of soil fertility the whole system of soil, soil solution, and plant is so constituted that all the components must be considered as interrelated. Thus, the plant may exhaust the soil solution with a resultant change in physical condition of the soil which may be unfavorable to the growth of microorganisms, and this inhibition in time may influence the concentration of certain solutes in the soil solution. It is believed that the greatest advances in theories of soil fertility will come with an extension of our knowledge of the soil solution in its dynamic aspects.

CONCLUSIONS

The physical state of certain soil constituents is influenced to a marked degree by the concentration of the soil solution. The colloidal state of the soil suspension undergoes significant alterations during the season. A large increase in colloidal matter is noted when the soil solution is depleted as a result of absorption of solutes by the plant.

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CARBON-DIOXID CONTENT OF BARN AIR

By MARY F. HENDRY and ALICE JOHNSON, *Carnegie Nutrition Laboratory, Boston, Mass., and New Hampshire Agricultural Experiment Station*

In connection with the construction and establishment of a respiration chamber [1] for large domestic animals in the dairy barn at the Agricultural Experiment Station, Durham, N. H., the question as to the carbon-dioxid content of barn air and its probable influence upon respiration experiments, in case such air should inadvertently leak into the chamber, assumed considerable importance. Recent information with regard to the carbon-dioxid content of barn air is extremely scarce, and the earlier work is practically unrecognized. The extensive investigations of Pettenkofer on ventilation, unfortunately published in a number of small and wholly inaccessible journals, have been cited from time to time by various writers, and to him have been ascribed carbon-dioxid percentages in stable air of 0.105 and 0.21 per cent. The most extended serious study of the carbon-dioxid content of barn air was that made by Schultze in the experiment station at Göttingen-Weende, the results of which have been reported by Märcker.[2] Employing the Pettenkofer method, Schultze made nearly 200 analyses of stable air in the vicinity of Göttingen and found that the carbon-dioxid content varied enormously, depending upon the number of animals in the stable, the volume of space available, and the degree of ventilation. The values for the carbon-dioxid percentages in the air of stables at Weende are as high as 0.435 per cent in a number of instances, and a maximum of 0.594 per cent is recorded. For outdoor air the usual value of not far from 0.03 per cent to 0.034 per cent was found. Märcker concludes that the ventilation of a stable should be such that the carbon dioxid in the air is not greater than 0.25 to 0.30 per cent. Angus Smith cites two analyses of the carbon-dioxid content of air in stables showing but 0.0833 and 0.0875 per cent.[3]

After our analyses of the air in the dairy barn at Durham were made, our attention was called to the report of the Committee on Farm

[1] BENEDICT, F. G., COLLINS, W. E., HENDRY, Mary F., and JOHNSON, Alice. A RESPIRATION CHAMBER FOR LARGE DOMESTIC ANIMALS. N. H. Agr. Exp. Sta. Tech. Bul. 16, 27 p., 7 fig. 1920.

[2] MÄRCKER, Max. UEBER DEN KOHLENSÄURE-GEHALT DER STALLLUFT UND DEN LUFTWECHSEL IN STALLUNGEN. *In* Jour. Landw., Jahrg. 17 (F. 2, Bd. 4), p. 224–275. 1869. We have seen this remarkably complete paper cited but once and then erroneously. It deserves careful study.

[3] SMITH, R. A. AIR AND RAIN. p. 50. London, 1872.

Building Ventilation.[1] In this report are given the results of analyses of air samples taken at various points in five different barns. Mr. Clarkson has called our attention to the fact that although the amounts of carbon dioxid per 10,000 parts of air are correctly expressed in the tables, the conversions to percentages are erroneous, because of misplaced decimal points, and the percentage values should accordingly be multiplied by 10. The results published in this report show that in the five barns examined, which were presumably of reasonably modern construction, the carbon-dioxid content of the air might be as high as 1.231 per cent, but for the most part was not higher than 0.2 to 0.3 per cent.

The dairy barn at Durham is admirably lighted and is, so far as one can judge by the senses at least, well ventilated. The stock room is approximately 100 feet long, 35 feet wide, and 8 feet 8 inches high, is provided with windows on both sides, and has a concrete floor. The ventilating ducts withdraw the air from near the floor, and outdoor air can blow in on either side through screened openings. Practical experience indicates that this barn is admirably adapted for maintaining stock in good health with a negligible amount of disease.

Our study of the air in this barn did not include an examination of the ventilation conditions, so far as draft, temperature, and psychrometric measurements are concerned, but consisted solely of gas analysis made in connection with the possibility of leakage of barn air into the respiration chamber. To study the carbon-dioxid content of the air in a modern, well-ventilated dairy barn seemed a justifiable procedure. Being unfamiliar at the time of our tests with the earlier series of observations cited above, we were astonished at our first results, which showed on the average an amount of carbon dioxid in the barn air not far from 8 to 10 times the normal carbon-dioxid content of outdoor air. The analyses were all made with the small Haldane gas analysis apparatus[2] by both authors at different times and after many years' experience with the use of this type of apparatus.[3]

To obtain a general picture of the distribution of the carbon dioxid in the air, samples were taken at different parts of the barn, but unfortunately not simultaneously. Four samples were taken at 8.50 a. m., four at 10.05 a. m., four at 11 a. m., and three at 11.40 a. m., all in different locations. Subsequently the samples were taken at three positions only, but variations in the time of day were studied under these conditions.

Approximately 40 milch cows were in the barn at the time. Of the 15 different positions at which air samples were taken, locations 1 to 5 were in the feed alley between the two rows of stalls and therefore in

[1] CLARKSON, W. B., SMITH, L. J., and IVES, F. W. [REPORT OF THE] COMMITTEE ON FARM BUILDING VENTILATION. *In* Trans. Amer. Soc. Agr. Engin. Rpt. 12th Ann. Meeting, 1918, p. 282–306, illus. 1919.

[2] HALDANE, J. S. METHODS OF AIR ANALYSIS. ed. 2, p. 68. London, 1918.

[3] Special mention should be made here of the intelligent cooperation in our work of the dairyman, Mr. Mario Quaregno, who collected samples for us at night with the greatest fidelity.

front of the animals. Locations 6 to 15 were in the two outer alleys and therefore at the rear of the animals. Locations 1 and 6 were nearest the respiration chamber. The results of the analyses are presented in Table I. All samples were taken approximately 4 feet from the floor.

TABLE I.—*Carbon dioxid in air of barn at Durham, N. H., during January and February, 1919*

Time of day.	Location.	Percentage of carbon dioxid.
8. 50 a. m.	1, beginning of feed alley	0. 228
Do.	2, feed alley, about 15 feet from No. 1	. 225
Do.	3, center of feed alley	. 214
Do.	4, feed alley, about 15 feet from No. 3	. 228
10. 05 a. m.	5, end of feed alley	. 194
Do.	6, beginning of right-hand outer alley [1]	. 106
Do.	7, outer alley, about 15 feet from No. 6	. 089
Do.	8, center of right-hand outer alley	. 098
11. 00 a. m.	9, outer alley, about 15 feet from No. 8	. 101
Do.	10, end of right-hand outer alley	. 097
Do.	11, beginning of left-hand outer alley	. 107
Do.	12, outer alley, about 15 feet from No. 11	. 089
11. 40 a. m.	13, center of left-hand outer alley	. 162
Do.	14, outer alley, about 15 feet from No. 13	. 116
Do.	15, end of left-hand outer alley	. 115
4. 45 p. m.	1, beginning of feed alley	. 149
5. 20 p. m.	2, feed alley, about 15 feet from No. 1	. 219
5. 30 p. m.	3, center of feed alley	. 207
5. 00 a. m.	1, beginning of feed alley	. 177
10. 30 p. m.	do	. 132
5. 00 a. m.	do	. 109
Do.	do	. 101
9. 30 p. m.	do	. 211
11. 30 p. m.	3, center of feed alley	. 102
5. 00 a. m.	do	. 211
10. 40 p. m.	do	. 187
5. 00 a. m.	do	. 167
11. 30 p. m.	do	. 184
5. 00 a. m.	do	. 130
11. 20 p. m.	do	. 139
5. 00 a. m.	do	. 168
11. 15 p. m.	do	. 178
5. 00 a. m.	do	. 094
11. 50 p. m.	do	. 209

[1] This position was nearest the respiration chamber.

Since under the conditions of experimentation the amount of carbon dioxid inside the respiration chamber varies from 0.1 to 0.7 per cent, being usually not far from 0.35 to 0.40 per cent, and since the method of experimentation depends upon the supplying of pure outdoor air with a carbon-dioxid content of 0.03 per cent, it can be seen that any leakage of barn air into the respiration chamber would be detrimental to the success of the experiment. The fact that all the control tests of this respiration chamber have shown most satisfactory agreement of results, when the technic is properly carried out, testifies to the care with which this chamber was constructed by the mechanician, Mr. W. E. Collins.

The production of carbon dioxid by dairy cows is very large because of several factors, among others the high metabolism of the animal itself and the conversion of carbohydrate into fat, which of itself results in a large splitting off of carbon dioxid (so-called "atypical" carbon dioxid). While the cows are not given any exercise when in the barn they are very energetic during feeding periods, striving to gather in every particle of food. At other times they are, for the most part, extraordinarily quiet and placid.

It is clear from the table that even in this modern barn there is a large percentage of carbon dioxid in the air. That the presence of this amount of carbon dioxid has, for two decades, had no apparent influence upon the health of the animals is worthy of special notice. The excellent health of the animals in this barn leads us to believe that what is true of men is likewise true of animals—that is, that carbon dioxid per se, even in percentages 8 or 10 times the normal percentage, has no serious effect upon the animal itself.

RICE WEEVIL, (CALANDRA) SITOPHITUS ORYZA

By RICHARD T. COTTON, *Scientific Assistant, Stored-Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

As early as 196 B. C. mention is made of the ravages of weevils in stored wheat (*9*).[1] Whether the species referred to was *Sitophilus oryza* L. or the closely allied granary weevil *S. granarius* L. we do not definitely know. The latter species, however, is thought to be the older and is presumably the one referred to. However that may be, since about the middle of the eighteenth century, when it was discovered in Europe, *S. oryza* has everywhere attracted the attention of scientists, and innumerable accounts have been written concerning its ravages. It is not the purpose of the writer to review at this time the extensive literature relating to this weevil; it will suffice to state that the early accounts are very general in character and the majority of the later ones little more than repetitions of the earlier observations. The publication of Hinds and Turner (*6*) in 1911 on the biology of the rice weevil gives us the only really definite information that we had regarding the life and habits of this species. A general presentation of the economic problem centered in the rice weevil was given in 1919 by Back (*1*) in a publication of the Department of Agriculture. It is with the purpose of adding to our knowledge of this cosmopolitan insect that this paper is presented.

ORIGIN AND DISTRIBUTION

The rice weevil, *Sitophilus oryza*, so called because of its discovery breeding in rice, is thought to have originated in India. It was carried by commerce to Europe at an early date, where it was subsequently found and described by Linnaeus in 1763 (*7, p. 395*).

At present it is perhaps the most widely distributed of known insects, being found in all parts of the world where grain is used. In North America it is reported from Florida to Alaska, though it is found in its greatest abundance in the South Atlantic and Gulf States.

DAMAGE CAUSED

From time immemorial the rice weevil has taken its yearly toll of the grain crops of man. The total amount of rice, corn, wheat, barley, rye, etc., that has been destroyed by this weevil alone is almost beyond conception.

[1] Reference is made by number (italic) to "Literature cited," p. 422.

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In the eight southern States of North America where the weevil is most abundant and destructive, 350,000,000 bushels of corn were produced in the year 1918. Of this vast amount it is estimated that approximately $28,000,000 worth was destroyed by the rice weevil alone. This represents only a small portion of the annual world crop of corn and a considerably smaller portion of the world crop of grains that are attacked by this weevil.

To cite another instance of the ravages of this weevil, Fitch (*2*) records that from 145 tons of American corn, 1¾ tons of weevils were screened out or, in round numbers, about 4,056,729,600 weevils, a truly enormous number. Such an occurrence as this was by no means rare in earlier times when cargoes of grain were transported long distances in slow-going vessels; in fact, it was not uncommon for whole cargoes to be destroyed by the weevil or rendered unfit for use.

At present losses are particularly severe in India, Mexico, South America, and other tropical countries where the weather conditions are such that the weevil can breed unchecked the year round.

Loss is occasioned by the feeding activities of both the grubs or larvæ and the adult beetles. The feeding of the larvæ is confined chiefly to the seeds of our common grains, but the adults feed on a great variety of seeds, fruits, and other foodstuffs. In addition to the loss in weight caused by the feeding of the larvæ and weevils, infested grain is often rendered unfit for consumption and has poor powers of germination.

FOOD OF ADULT WEEVILS

The adult weevils feed on a great variety of seeds and seed products. The following list has been compiled from the numerous reports of the feeding habits of this weevil: Rice, wheat, corn, barley, rye, hulled oats, buckwheat, maize, chickpeas, table beans, millet, chestnuts, cashew nuts, bird seed, seed of *Nebulium* sp., hemp seed, Job's tears (*Coixa lachryma*), packages of "feuilles de sagon," packages of cereals, tobacco, peaches, grapes, apples, mulberries, bags of meal, yeast cakes, biscuits, macaroni, cakes, crackers, wheat flour, rice flour, and white bread and other wheat products. The author has found the adult weevils burrowing and feeding in the berries of the Chinaberry tree, in both Irish and sweet potatoes, and in the seed of the avocado. In the laboratory they showed a liking for most kinds of ripe fruits, and it was found that they would live indefinitely on a majority of the wild berries growing in the vicinity of the laboratory. With such adaptable food habits as this long list would indicate it is little wonder that this weevil is so widespread and causes so much damage.

FOOD OF LARVÆ

The larvæ or grubs of the rice weevil are much more restricted in their diet than are the adult beetles, owing to the fact that they pass the entire larval period within a single seed and are limited to seeds that contain

sufficient food to enable them to develop to maturity. They have been reported to breed in rice, wheat, corn, hulled oats, millet, barley, rye, buckwheat, chickpeas, Job's tears (*Coixa lachryma*), acorns of several species of oak, galls of *Phylloxera devastatrix* on *Hicoria pecan*, and old cotton bolls.

LIFE HISTORY

The observations from which the following data are taken were made in Orlando, Fla., during the year 1919 and the early part of 1920. Since this weevil is of more importance in the southern States as a pest of corn, the life-history records were taken from weevils breeding in corn.

All stages of the rice weevil are active throughout the year in Florida. The egg, larval, and pupal stages are somewhat prolonged during the winter months, but there is no hibernation period, and oviposition continues throughout the year.

The adult weevils appear on corn in the field as soon as it reaches the roasting-ear stage and are often to be found in the markets at this time on the ears presented for sale. It is not until the corn has become a little firmer, however, that oviposition begins. When it has reached the firm stage the female weevils oviposit in all parts of the grain that can be reached with the proboscis and ovipositor, for at this time it is a simple matter for the weevil to excavate an egg cavity, and the rate of oviposition is much greater at this time than later when the corn has hardened. As the kernels of corn become harder the majority of the eggs are laid in the white starch part of the kernel that is found at the outer end as the kernel is attached to the cob. With shelled corn the majority of the eggs are deposited in the soft germ part near the tip of the kernel where excavation is relatively easy.

In the field the ears with tips protruding from the shucks, those with loose, open shucks, or those with shucks that have been injured by the corn earworm or some other agency are the first to be infested. Ears that have a long, tight-fitting shuck that extends well beyond the tip of the ear at the period when the corn is ripening are practically immune from weevil attack. The weevils encounter great difficulty in penetrating a well-developed, tight-fitting shuck and therefore congregate on the ears with the damaged or poorly developed shucks. The kernels at the exposed tip are the first to be infested, but the weevils soon work their way to all parts of the ear.

METHOD OF OVIPOSITION

The female weevil after selecting a favorable spot on a kernel of corn proceeds to excavate the egg cavity. This she accomplishes with her powerful though slender proboscis or beak, oscillating her body in such a manner as to impart a combined up and down and rotary motion to the proboscis. The mandibles attached at the end of the beak chew away at the corn until finally a hole is excavated equal to the length of the

proboscis. The cavity is trimmed and enlarged and the sides smoothed off until the weevil is satisfied that all is as it should be. She then withdraws her proboscis and turning around swings the abdomen about until the egg cavity is located. The ovipositor is then thrust into the cavity and a single egg is deposited.

Before the ovipositor is withdrawn a translucent mass of material is discharged on top of the egg and is tamped down level with the surface of the kernel of corn, forming a protective cap to the egg. This cap, because of its translucent character, assumes the color of the portion of the kernel in which it is located, thereby making the discovery of the egg difficult at times. Occasionally one or more extra discharges are made on top of the first cap, causing the cap to protrude above the surface of the kernel. These latter discharges are usually irregular, opaque, and mixed with fecal matter.

The time taken to excavate the egg cavity varies with the condition of the grain. When the corn is soft the cavity may be completed in less than 30 minutes, whereas in hard corn the operation may take as long as 2 hours. The actual time of depositing the egg after the cavity is finished is short, from 3 to 4 minutes on the average.

WHERE THE EGGS ARE PLACED

The egg cavities are made usually in some part of the soft starch of the grain or in the germ, where the work of excavation is easier and the young larva upon hatching will have an abundance of food ready for instant use. Frequently in kernels of corn that have not sufficiently hardened numerous excavations will be made only to be abandoned by the weevil as unfit for use, the weevils apparently having the instinct of knowing when the corn is unfit to maintain larval life. Several eggs are often deposited in the same kernel of corn, though when the supply of grain is abundant it is not usual for a weevil to deposit more eggs in a single kernel than can mature in the limited amount of food present. When weevils are confined with only a few kernels, however, the instinct to continue laying eggs predominates and eggs are deposited in all parts of the grain.

The egg itself is somewhat flexible in character and conforms to the shape of the egg cavity. It is placed with the top just below the surface of the kernel and with the larger end toward the inner end of the cavity.

RATE OF OVIPOSITION

The rate of oviposition varies with the condition of the grain, the age of the weevil, and the temperature. During the warm weather of summer, with young female weevils and with corn in the "hard gum" stage, the oviposition rate reaches its maximum. Under such conditions from 8 to 10 eggs are laid per day, though as many as 20 to 25 may occasionally be laid in a like period.

As the weevils get older the oviposition rate gradually decreases until, a few weeks before death, egg laying ceases altogether. With the approach of cold weather the rate of oviposition also decreases, and especially is this true of the older weevils. The younger female weevils are more vigorous and are much less affected by the cold.

Normally eggs are laid every day during the spring, summer, and fall months, but during the winter egg laying is sporadic and is controlled chiefly by the daily temperatures.

In Florida the winter temperatures are very variable, short periods of cold weather occur frequently, and during these oviposition usually ceases.

During the warmer months the weevils normally lay from three to six eggs per day in hard corn.

Table I shows the rate of oviposition at various times of the year. It contains abstracts from the oviposition records of 14 weevils that are representative of the species. The number of eggs laid by each weevil on two consecutive days in each week from June, 1919, to March, 1920, is given, together with the daily mean temperatures and the dates of emergence and death of each individual weevil. The corn was at its most favorable stage for oviposition during the latter part of June and the early part of July.

TABLE I.—*Rate of oviposition of Sitophilus oryza; extracts from oviposition records at Orlando, Fla., June, 1919, to March, 1920*

Date.	Mean temperature.	Number of eggs laid by weevil No.—													
		A_2	A_3	B_2	B_6	C_3	C_7	D_1	D_2	D_4	E_3	E_6	F_1	F_2	F_4
1919.	°F.														
June 22	82.5	10	5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
23	80	5	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28	78.5	12	7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
29	79.5	13	8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
July 3	79	11	16	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4	78.5	11	23	(a)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	81	12	15	9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	82.5	14	11	13	(a)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
17	81.5	6	12	8	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
18	81.5	6	9	5	8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
25	81	1	(b)	5	6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
26	81	3	.....	6	5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Aug. 4	85	(b)	.....	6	8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	85.5	.....	.....	9	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	81.5	.....	.....	7	7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	83	.....	.....	8	10	(c)	.....	.....	.....	.....	.....	.....	.....	.....	.....
18	81.5	.....	.....	8	10	.....	(d)	.....	.....	.....	.....	.....	.....	.....	.....
19	81.5	.....	.....	7	6	2	.....	.....	.....	.....	.....	.....	.....	.....	.....
27	82.5	.....	.....	7	5	8	3	.....	.....	.....	.....	.....	.....	.....	.....
28	82	.....	.....	8	7	7	4	.....	.....	.....	.....	.....	.....	.....	.....
Sept. 3	80.5	.....	.....	4	4	7	4	.....	.....	.....	.....	.....	.....	.....	.....
4	81.5	.....	.....	6	8	8	2	.....	.....	.....	.....	.....	.....	.....	.....
11	84	.....	.....	4	7	6	6	.....	.....	.....	.....	.....	.....	.....	.....
12	84	.....	.....	3	7	4	4	.....	.....	.....	.....	.....	.....	.....	.....
19	82.5	.....	.....	6	5	5	6	.....	.....	.....	.....	.....	.....	.....	.....
20	83	.....	.....	4	5	6	6	(e)	(e)	(f)	.....	.....	.....	.....	.....
28	77.5	.....	.....	3	4	5	4	4	3	4	.....	.....	.....	.....	.....
29	73.5	.....	.....	3	4	4	4	3	4	5	.....	.....	.....	.....	.....

a Weevil emerged July 5, 1919.
b Beetle escaped.
c Weevil emerged Aug. 10, 1919.
d Weevil emerged Aug. 18, 1919.
e Weevil emerged Sept. 18, 1919.
f Weevil emerged Sept. 19, 1919.

TABLE I.—*Rate of oviposition of Sitophilus oryza; extracts from oviposition records at Orlando, Fla., June, 1919, to March, 1920*—Continued

Date.	Mean temperature.	Number of eggs laid by weevil No.—													
		A_2	A_3	B_2	B6	C_3	C_7	D_1	D_2	D_4	E_3	E6	F_1	F_2	F_4
1919.	°F.														
Oct. 1	77.5			6	3	6	6	4	6	6					
2	82.5			4	3	6	5	4	7	5					
10	81			2	2	2	2	5	5	4					
11	80			2	2	1	2	5	5	4					
19	80			1	3	4	4	5	5	5					
20	80.5			1	2	3	4	4	5	6					
26	78.5			(*g*)	1	3	4	4	5	2					
27	76.5				1	2	3	3	4	2					
Nov. 3	76.5				2	3	2	4	5	5					
4	76				2	3	2	3	5	4	(*h*)				
10	70.5					2		3	2	1	3				
11	70				1	1		2	2	3	5				
17	70							2	1	2	3				
18	59				1	1						(*i*)			
26	69						(*j*)	2	1	1	3	3			
27	70.5								1	1	5	3			
Dec. 3	71.5				1			4	1		3	1			
4	67							1	2	1	2	2	(*k*)		
10	71								1		4	4	1		
11	76							2	3	3	5	4	1		
20	61.5								1		1	1			
21	67							2	1		5	2			
27	59							2							
28	57				(*l*)						1	1			
1920.															
Jan. 3	57										1				
4	45.5														
9	61.5														
10	68							1			2	1	1		
20	62											2		(*m*)	
21	65.5													1	
29	70							1			2			2	(*n*)
30	69								3		2	5		2	3
Feb. 3	65.5														
4	64.5							3	2	2	2	5		1	3
12	68							1	1		2	2	1	3	1
13	71										2	3		2	
21	61.5													1	1
22	63.5								1			1		4	1
25	62.5								1		1	1		1	1
26	54												1	1	1
Mar. 1	41.5														
2	44				(*o*)			(*p*)	(*o*)	(*q*)	(*o*)	(*o*)	(*o*)	(*o*)	(*o*)

g Female died Oct. 25, 1919.
h Weevil emerged Oct. 30, 1919.
i Weevil emerged Nov. 6, 1919.
j Weevil died Nov. 26, 1919.
k Weevil emerged Nov. 30, 1919.
l Female died Dec. 30, 1919.
m Weevil emerged Dec. 9, 1919.
n Weevil emerged Dec. 15, 1919.
o Female living.
p Female died Mar. 5, 1920.
q Female died Feb. 25, 1920.

NUMBER OF EGGS LAID

The largest number of eggs laid by a single weevil was 576. These were laid during a period of 149 days. The weevil in question emerged July 5, 1919, began laying eggs on July 12, and continued oviposition until December 7, 1919. Egg laying was apparently stopped by the cold weather and the exhaustion of the weevil, and death occurred December 30, 1919. This oviposition record is in all probability longer than the average, though it does not represent the maximum period, for when winter intervenes, a period during which few eggs are laid, the oviposition period may be considerably longer.

Table II contains data concerning the preoviposition period, the oviposition period, and the number of eggs laid. The records of the 10 individuals cited were selected as being representative.

TABLE II.—*Data concerning oviposition and longevity of Sitophilus oryza at Orlando, Fla., 1919*

Weevil No.	Date weevil emerged.	Date first egg was laid.	Length of preoviposition period.	Date last egg was laid.	Length of oviposition period.	Number of eggs laid.	Date of death.	Length of life.
			Days.		*Days.*			*Days.*
1	July 3	July 9	6	Oct. 5	89	270	Oct. 5	95
2	5	9	4	24	108	552	25	113
3	5	12	7	Dec. 7	149	576	Dec. 30	179
4	5	16	11	Oct. 3	80	288	Oct. 5	93
5	8	15	7	Sept. 19	67	420	Sept. 23	78
6	14	21	7	Nov. 7	110	445	Nov. 20	130
7	18	24	6	Oct. 22	91	339	Oct. 23	98
8	Aug. 10	Aug. 19	9	Nov. 5	79	237	Nov. 28	111
9	10	19	9	18	92	389	Dec. 6	119
10	18	26	8	7	74	284	Nov. 26	101
Average			7.4		93.9	380		111.7

From Table II it will be seen that the average preoviposition period is about 7 days, the average oviposition period during the warm months of the year is 93.9 days, and the average number of eggs laid per female is 380, or about 4 per day.

DESCRIPTION OF EGG

Egg opaque, shining, white, ovoid to pear-shaped in form, widest below middle, bottom broadly rounded, neck narrowing sharply towards top, which is somewhat flat and bears a small protuberance that fits into a cap or plug which cements the egg into place. Length 0.65 to 0.70 mm.; width 0.28 to 0.29 mm.

INCUBATION PERIOD

The eggs usually hatch in from 3 to 5 days during the warm months of the year, although by far the majority of them hatch in 4 days. During the colder weather of winter the incubation period is somewhat longer and may last 10 or more days. The variation in the length of the incubation period at different times of the year may be seen in Table III.

LARVAL PERIOD

The embryo develops within the egg with its head toward the top, the darker color of the mandibles showing through the thin, transparent shell some time before the egg hatches. The eggshell undulates with the movements of the newly formed larva but is finally ruptured and the young larva begins to feed on the tissues of the corn.

The egg is usually placed so that at least part of it is embedded within the soft white starch of the grain so that the young larva is at once supplied with a readily available food supply. Occasionally the egg is surrounded entirely by the horny portion of the seed, and in this case growth of the larva is somewhat slower until it makes its way to the softer white part.

TABLE III.—*Life-history data on Sitophilus oryza, 1919–20*

Weevil No.	Date egg was laid.	Date egg was hatched.	Length of egg stage.	Date of first molt.	Length of first larval stage.	Date of second molt.	Length of second larval stage.	Date of third molt.	Length of third larval stage.	Date prepupal form appeared.	Length of fourth larval stage.	Date pupated.	Length of prepupal stage.	Date adult emerged.	Length of pupal stage.
			Days.		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*
1	May 10	May 14	4	May 19	5	May 23	4	May 28	5	June 4	7	June 5	1	June 12	7
2	12	17	5	21	4	26	5	31	5	8	8	9	1	15	6
3	June 6	June 10	4	June 14	4	June 19	5	June 23	4	July 1	8	July 2	1	July 7	5
4	8	11	3	15	4	19	4	22	3	June 29	7	June 30	1	5	5
5	10	15	5	19	4	23	4	27	4	July 3	6	July 4	1	9	5
6	11	16	5	20	4	24	4	28	4	6	8	7	1	13	6
7	July 2	July 6	4	July 10	4	July 14	4	July 18	4	23	5	24	1	29	5
8	2	6	4	10	4	14	4	18	4	24	6	25	1	30	5
9	3	7	4	11	4	15	4	19	4	23	4	24	1	28	4
10	3	7	4	10	3	14	4	18	4	26	8	27	1	Aug. 1	5
11	4	8	4	11	3	15	4	18	3	27	9	28	1	2	5
12	Aug. 4	Aug. 7	3	Aug. 11	4	Aug. 14	3	Aug. 18	4	Aug. 27	9	Aug. 28	1	Sept. 1	4
13	4	8	4	12	4	15	3	19	4	25	6	26	1	Aug. 30	4
14	5	9	4	13	4	17	4	21	4	29	8	30	1	Sept. 4	5
15	10	13	3	17	4	21	4	25	4	30	5	31	1	5	5
16	Sept. 2	Sept. 6	4	Sept. 10	4	Sept. 14	4	Sept. 18	4	Sept. 24	6	Sept. 25	1	30	5
17	10	14	4	17	3	21	4	25	4	Oct. 1	6	Oct. 2	1	Oct. 7	5
18	17	20	3	24	4	27	3	Oct. 2	5	10	8	11	1	16	5
19	27	Oct. 2	5	Oct. 6	4	Oct. 11	5	15	4	20	5	21	1	26	5
20	Oct. 2	6	4	10	4	13	3	17	4	24	7	25	1	30	5
21	8	12	4	16	4	20	4	25	5	30	5	31	1	Nov. 5	5
22	17	22	5	26	4	31	5	Nov. 5	5	Nov. 14	9	Nov. 15	1	21	6
23	28	Nov. 2	5	Nov. 6	4	Nov. 10	4	15	5	23	8	24	1	30	6
24	Nov. 2	7	5	11	4	16	5	23	7	Dec. 10	17	Dec. 12	2	Dec. 22	10
25	8	14	6	19	5	25	6	Dec. 1	6	19	18	20	1	29	9
26	15	25	9	Dec. 4	9	Dec. 12	8	23	11	Jan. 12	20	Jan. 14	2	Jan. 26	12
27	25	Dec. 1	6	12	11	22	10	Jan. 3	12	19	16	21	2	31	10
28	Dec. 2	8	6	20	12	30	10	10	11	27	17	29	2	Feb. 8	10
29	6	12	6	24	12	Jan. 6	13	22	16	Feb. 13	22	Feb. 15	2	Mar. 6	20
30	14	22	8	Jan. 3	12	14	11	26	12	13	18	15	2	6	20

DESCRIPTION OF LARVA

Mature larva from 2.5 to 3 mm. in length. A pearly white, fleshy grub, very thick-bodied, the ventral outline being approximately straight while the dorsal outline is almost semicircular. Head light brown in color, the anterior margin and mandibles much darker. Head longer than broad and somewhat wedge-shaped, the sides broadly rounded from middle to apex, which is slightly angular. Sides nearly straight from middle to the anterior angles, and lateral area with an oblique, longitudinal, lighter stripe or area. Epicranial and frontal sutures distinct and light in color; also two oblique, longitudinal, light stripes rising from frontal sutures and coalescing with epicranial suture near base of head. Frons subtriangular with a distinct, dark, median line indicating the carina running from the posterior angle to beyond the middle. Sutural margins irregular or sinuate. Frons provided with five pairs of large setæ, the sutural margins each bearing a large seta. Each epicranial lobe bearing the following setæ: One close to posterior angle of frons and located within the oblique, longitudinal stripe rising from the frontal suture; one very small seta posterior to this and near occiput; two anterior to it on disk of epicranium; two opposite middle of frons; one opposite middle of mandible; one opposite hypostomal angle of mandible; and one on hypostoma near base of mandible. Epistoma represented by thickened anterior margin of front, distinctly darker in color, with anterior margin declivous and slightly curving and lateral angles slightly produced and elevated where they support the dorsal articulation of the mandibles. Pleurostoma represented by the darker declivous area surrounding the mandibular foramen. Mandibles stout, triangular, with the apex produced into a broad apical tooth; inner edge toward the apex provided with a subapical tooth and a small medial tooth; no molar part. Dorsal area of mandible provided with a pair of stout bristles set apart. Eye represented by a well-defined black spot beneath the exoskeleton. Clypeus attached in front of frons and broadly transverse, broad at base, sides narrowing toward the apical angles, slightly longer and broader than labrum, and bearing on epistomal margin two fine setæ on each side. Labrum distinctly broader than long, with two small lateral and a larger, rounded, median lobe. Labrum provided with six large setæ behind middle, two marginal, short, thickened setæ on each lateral lobe, and six similar marginal setæ on median lobe.

Maxilla with cardo present and distinct, stipes not divided into stipes proper, subgalea, and palpifer but one continuous piece, with the anterior inner angle produced into a single setose lobe. Palpus 2-jointed, bearing a single seta near apex of first segment. There are three other setæ on maxilla, two located on the vaginant membrane between palpus and palpifer and one stouter and longer midway between palpus and cardo. No articulating maxillary area between maxilla and mentum-submental region.

Labium: Submentum and mentum fused and represented by a broad lobe bearing three pairs of stout setæ. Stipes labii posteriorly enforced by a median, triangular chitinization, the anterior, median section produced anteriorly between the palpi into a small lobelike ligula which is fused with the lingua. Each stipes labii bearing a single seta. The short, conical, 2-jointed palpi are situated on the anterior angles of the stipites. The ligula bears four small setæ.

Prothorax dorsally not divided, but two areas, praescutal and scuto-scutellar, are roughly indicated by rows of setæ. The mesothoracic and metathoracic segments are above divided into two distinct areas, the anterior of which represents the praescutum and the posterior the scuto-scutellum and alar area. The thoracic spiracle is located on a lobe pushed into the prothorax from the epipleurum of the mesothorax. It is bifore, elongate, larger than abdominal spiracles, and placed with the fingerlike air tubes pointing dorsad.

Ten abdominal segments; ninth small, tenth reduced. Each tergum of the first three abdominal segments is above divided into three distinct areas, praescutum, scutum, and scutellum. Each tergum of the fourth to eighth abdominal segments is above divided into but two areas, the first of these containing the praescutal and scutal elements, the second representing the scutellum. Below these two areas and adjacent to the epipleurum is the alar area. The abdominal spiracles are placed anteriorly and in a small, separate corner piece probably of the alar area; they are bifore and are found on abdominal segments 1 to 8, that on the eighth being located slightly more dorsad than the rest. Below a very indistinct and abrupt dorso-lateral suture and above a well-defined ventro-lateral suture is a large, not subdivided epipleurum. The abdominal epipleura are located considerably higher than the thoracic lobes. Below the ventro-lateral suture is the hypopleurum, subdivided into three lobes, one right under the other. Below the hypopleurum is the coxal lobe, and below that is the sternum, consisting of eusternum and a posterior triangular area representing the parasternum or parasternum fused with sternellum. Abdominal segments provided with setæ as follows: One on praescutum, a long and two short ones on scutellum, two on alar area located just above spiracle, two on epipleurum, one on coxal lobe, and two on eusternum. One of the setæ on scutellum is usually missing on abdominal segments 5 to 9.

LARVAL STAGES

First-stage larva: Similar in appearance to mature larva but smaller; width of head capsule 0.22 mm.

Second-stage larva: Width of head capsule 0.32 mm.

Third-stage larva: Width of head capsule 0.48 mm.

Fourth-stage larva: Width of head capsule 0.64 mm.

NUMBER OF LARVAL STAGES

After hatching the larva feeds rapidly, molting three times at more or less regular intervals. Previous writers have stated that there are only three larval stages. This is erroneous; there are invariably four, as is the case with other weevils of this genus. Owing to the fact that the larva passes its entire existence buried within the seed and obscured from view it is somewhat difficult to observe all the changes that take place. The writer, however, with the aid of binoculars and dissecting instruments has followed through the life histories of several hundred individuals at various times of the year, making daily observations on each individual.

The first three larval stages average four days each, while the fourth stage varies from four to nine days. During the cooler weather the periods are all lengthened. Table III gives a good idea of the varying length of the larval stages at different times of the year.

LARVAL HABITS

The larva occasionally bores near the surface of the grain, forming elongate mines filled with white frass, but it more often bores directly down into the heart of the seed. As it feeds and moves along, the frass and débris are kept packed behind it. The space around it is kept

clear and free and is slightly larger than the grub, so that the latter can readily turn around if it desires. If it is disturbed, the grub will turn its head toward the point of attack, gnashing its mandibles.

PREPUPAL STAGE

When it is fully grown, the larva constructs a pupal cell. It uses the end of its burrow for this purpose, strengthening the weak and soft sides of the cavity with a cement formed from a larval secretion mixed with frass and waste material of the burrow. This forms a hardened shell around the larva. After it is completed the larva becomes sluggish, lengthens out, and loses its plump appearance. This prepupal stage invariably lasts for one day except during the winter months when it usually lasts for two days; then the pupal form is assumed.

PUPAL STAGE

The pupal stage normally lasts for five days. On the fourth day the mouth parts begin to color, then the tips of the inner wings. Spots of color show on the prothorax, the beak, and the appendages and finally on all parts of the body. On the fifth day the adult form is assumed.

DESCRIPTION OF PUPA

Pupa uniformly pearly white when first formed. Length 3.75 to 4 mm.; width about 1.75 mm. Tips of wing pads attaining seventh abdominal segment, tips of metathoracic tarsi extending beyond tips of inner wings. Head rounded, beak elongate and slender. Head with two prominent spines toward vertex, a group of two small spines and two spinules on each side above eyes, two pairs of small spines near anterior margin, and one on each side of front between the eyes. Three pairs of spines on beak between frontal ones and base of antenna, a pair of small ones on beak midway between base of antenna and tip of beak, a pair on sides of beak between latter pair and tip of beak, and two pairs of smaller ones on tip of beak.

Prothorax provided with one pair of anteromarginal setigerous tubercles, one pair of anterolateral, two pairs of mediolateral, and four pairs of dorsal setigerous tubercles.

Mesonotum and metanotum each provided with three pairs of spines.

Abdomen with seven distinct dorsal tergites, the seventh being much larger than the rest, dorsal area of each armed with a pair of large and a pair of smaller spines. Lateral area of each tergite armed with a spine, at the base of which is a small seta. Epipleural lobes each armed with two minute spines. Ninth segment as usual armed with two prominent spines.

ADULT

The mature weevil measures from 2.1 to 2.8 mm. in length and is a dull brown. It has the thorax densely pitted with round punctures, and the elytra are marked with four reddish spots.

The adult weevil on first transforming is soft and is light in color and stays within the pupal cell until it has hardened and become darker. It usually emerges from the grain within a few days after transforming

but may sometimes remain within to feed. In winter months individuals have been observed to remain within the grain for as long as a month before cutting their way out.

NUMBER OF MALES AND FEMALES

Males and females are apparently produced in very nearly equal numbers. Of 1,000 bred specimens examined 52 per cent were females and 48 per cent males. The majority of the specimens examined were bred during the later months of the year when the percentage of females produced was slightly higher. During the early months of summer more males were bred than females. Whether these conditions hold true always can not be determined until many more specimens have been reared and examined.

COPULATION

Copulation takes place within a day or two after emergence, one female weevil being observed in copula two days after assuming adult form. Copulation is frequent. It occurs rather often during the daytime but more frequently at night.

PARTHENOGENESIS

Unfertilized female weevils, as previously reported by Hinds and Turner (*2*), do deposit eggs that are fertile. The rate of oviposition is very much lower, however, than with fertilized females, and very few of the eggs hatch and produce grubs.

LIFE CYCLE AND NUMBER OF GENERATIONS

The period from egg to adult during the warm months of the year averages 28 days, which together with a preoviposition period of 7 days gives a life cycle of approximately 35 days. In some cases the life cycle is completed in a much shorter period, one reared individual completing the cycle in 30 days. On the other hand, the life cycle may be very considerably prolonged on account of unfavorable food and weather conditions.

Table III presents the life-history data of 30 weevils bred at various times of the year and shows the variation in the length of the stages from egg to adult at different seasons.

In Florida there are usually about seven full generations a year, six during the period from April to November and one from December to March.

MULTIPLICATION

Several calculations have been made and published of the theoretical number of the progeny of a single pair of weevils. Owing to lack of information on the rate of oviposition, the number of eggs laid, and the length of the life cycle, the number has in some cases been greatly underestimated and in other cases greatly overestimated. From the data given in Table II it is to be seen that the average female weevil lays

about four eggs a day for a period of nearly 100 days. Taking 35 days as the length of the average life cycle, we find that by the time the female weevil has stopped laying eggs, or in about three months' time, the progeny from a single pair of weevils would theoretically amount to approximately 100,000 weevils. From this time on during warm weather the increase would be extremely rapid and is left to the imagination of the reader.

LONGEVITY

The length of life of the adult weevils is variable and depends upon a number of different factors. With weevils that emerge during the spring and summer months the average length of life is from three to six months. In this case the weevils mate almost immediately after emergence, and egg laying ensues. The female weevils continue depositing eggs until exhausted and then die. With weevils that emerge in the fall and winter months, mating and oviposition are less frequent, the weevils do not become exhausted so rapidly, and life is consequently prolonged. Several female weevils that were kept segregated and were not allowed to mate laid only a few eggs, did not become exhausted, and were still alive eight months from the date of emergence. In another case several weevils of both sexes were kept segregated for a period of four months and were then allowed to mate. Of these, several weevils of both sexes were still alive and active eight months from date of emergence.

Weevils deprived of food do not live long. In cold weather when they are somewhat sluggish specimens have lived for 30 days without food. In warm weather, however, they are very active and soon become exhausted, seldom surviving for more than a week without food.

FEIGNING DEATH

When suddenly disturbed, the adult weevils often feign death, drawing their legs up close to the body and dropping. This state does not last long, and the weevils are soon hurrying off as active as ever. It is interesting to note that the habit of feigning death is not nearly so well developed in this species as it is in the closely allied species *Sitophilus granarius*. Weevils of the latter species feign death at the slightest disturbance and remain motionless for a considerable length of time. The fact that *S. oryza* possesses functional wings with which to escape, while *S. granarius* does not, may have some bearing on the explanation.

PARASITES

Parasites of *Sitophilus oryza* are numerous and attack all stages of this insect. A predaceous mite, *Pediculoides ventricosus* Newport, is often found in weevil-infested corn in the southern States and attacks and kills eggs, larvæ, and pupæ.

Two hymenopterous parasites, *Cercocephala elegans* Westwood and *Aplastomorpha vandinei* Tucker, are found in great abundance in Florida attacking the larvæ.

In addition to the parasites mentioned above, Pierce (*8, p. 80*) reports the following Hymenoptera as being parasitic on *Sitophilus oryza: Meraporus calandrae* Howard,[1] *M. utibilis* Tucker, [1] *M. requisitus* Tucker, and *Catolaccus incertus* Ashmead.

From Australia Mr. G. F. Hill (*5*) reports that he bred the two chalcids *Spalangiomorpha fasciatipennis* Girault and *Neocatolaccus australiensis* Girault [1] from grain infested with *Sitophilus oryza.* T. B. Fletcher (*3*) reports that the adult beetle *Tenebroides mauritanicus* L. preys upon adult weevils of *Sitophilus oryza.*

CONTROL MEASURES

Of the vast number of remedies that have been advocated for the control of this weevil the most effective agents now known are carbon disulphid and heat.

Infested grains should be fumigated in a gas-tight container or crib. Four to 6 pounds of carbon per 1,000 cubic feet used in such a crib has proved to be very effective in ridding the grain of the weevils.

Where it is practicable to apply heat to the infested grain, this method of control will prove very effective. A temperature of 116° F. maintained for two hours will kill all adults, and a temperature of 124° maintained for two hours will kill all stages from egg to adult.

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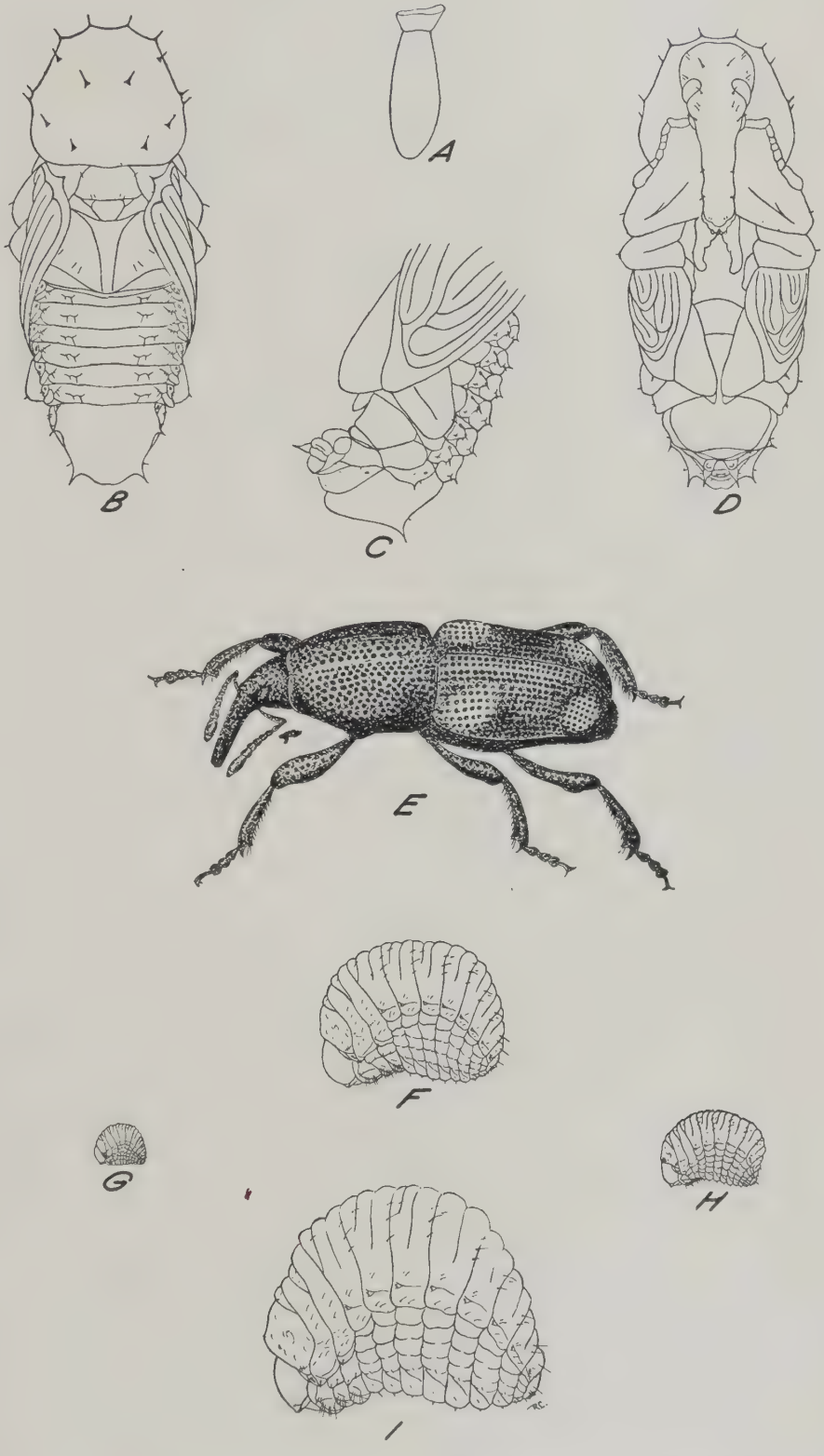
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196 B. C.? CURCULIO, OR THE FORGERY.

[1] Gahan (*4*) has pointed out that *Meraporus utibilis* Tucker and *Meraporus calandrae* Howard are both identical with *Lariophagus distinguendus* Foerster, and he also states that Girault has reduced *Neocatolaccus australiensis* Girault to synonymy with *Aplastomorpha vandinei.*

PLATE 60

Sitophilus oryza:

A.—Egg.
B.—Pupa, dorsal aspect.
C.—Pupa, lateral aspect.
D.—Pupa, ventral aspect.
E.—Adult.
F.—Third-stage larva.
G.—First-stage larva.
H.—Second-stage larva.
I.—Fourth-stage larva.


A
B
C
D
E
F
G
H
I

OPIUS FLETCHERI AS A PARASITE OF THE MELON FLY IN HAWAII

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INTRODUCTION

The braconid parasite *Opius fletcheri* Silvestri was introduced into the Hawaiian islands from India in May, 1916, by D. T. Fullaway, representing the Board of Commissioners of Agriculture and Forestry of the Territory of Hawaii. It was brought in as a parasite of the melon fly (*Bactrocera cucurbitae* Coquillett) which had been causing great losses to the vegetable growers of the islands. The only host here which it attacks freely under field conditions is the melon fly, although it can be bred freely in the laboratory from the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann). From many thousands of Mediterranean fruit-fly puparia, secured from fruits collected in the field, only four adult *O. fletcheri* have been reared. One was bred from fruit-fly larvæ developing in fruits of *Chrysophyllum oliviformae*, one from larvæ in fruits of tropical almond (*Terminalia catappa*), and two from larvæ secured from coffee (*Coffea arabica*). The first two were collected in Honolulu, and the last two were from the Kona district of the island of Hawaii.

A clear conception of the biology of this parasite and a record of its activities since its introduction into Hawaii are the two principal objects of this paper.

DESCRIPTION AND LIFE HISTORY

EGG

The egg is always deposited in the larva of the host, just beneath the skin. Its pointed, attenuated end becomes firmly glued to the inner surface of the larval integument by a dark, almost black substance; and its free end projects obliquely into the body cavity of the larva. The spot receiving the egg soon becomes darkened; and the dark substance by which the egg is attached to the host larva may be a darkened clot of larval fluids which originally exuded when the wound was made by the insertion of the ovipositor.

Immediately after deposition (fig. 1) the egg is cylindrical, bluntly pointed at both ends, slightly more convex dorsally than it is concave ventrally, and translucent white with a smooth, glistening surface. Its average length is 0.54 mm. and it is about one-sixth as broad as long. Just before hatching (fig. 2), its width is a little over one-third the length,

[1] Credit is due C. E. Pemberton, formerly with the Bureau of Entomology, for the drawings contained in this paper and for the greater part of the microscopic work performed during its preparation.

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which averages 0.66 mm., the cephalic end being drawn out into a distinct tubercle while the caudal end retains the blunt point. At this time magnification renders the embryo plainly visible.

Only by careful dissections of host larvæ into which many eggs of *Opius fletcheri* have been deposited during a short period is it possible to ascertain accurately the duration of the egg stage. In the month of July, 1918, 439 eggs were under observation, all of which hatched between 37 and 40 hours after oviposition. The eggs may hatch while the host is still a larva, or after it has formed a puparium. Even though a host larva contains several parasite eggs or newly hatched larvæ, it is not killed but continues to feed in an apparently normal manner and eventually leaves the fruit and forms its puparium. In fact, the parasite seems to have no effect upon the development of the fly until a complete histolysis of the larval tissues within the puparium has taken place. At this time all development of the parasitized fly ceases. No histogenesis occurs, and the young parasite larva develops rapidly by feeding upon the liquid mass of the broken-down larval tissues of its host which surround it.



FIG. 1.—*Opius fletcheri:* Egg just deposited. Length 0.54 mm.

LARVA

During this period of development there are four distinct instars, during which many interesting changes occur. The first instar (fig. 3) is easily distinguished by a large, chitinized head bearing the strong, pointed mandibles, and by the chitinized ventral plate of the head which has a distinct U-shaped cephalic line. In this stage a tracheal system is present, but no open spiracles can be seen, even with high magnification. The two longitudinal, lateral trunks throw out branches into each body segment, including the head, and are connected at their cephalic and caudal extremities by a transverse connecting branch. When first hatched, the larva is surrounded by a mass of egg serosal cells, which cling to it until it is almost ready to molt into the second instar. This mass, however, has never been observed clinging to the first larval molt (fig. 4), as it does in the case of the three Mediterranean fruit-fly parasites (*Opius humilis* Silvestri, *Diachasma tryoni* Cameron, and *D. fullawayi* Silvestri).[1] The digestive tract, which is a simple tube the greater portion of which consists of the large intestine, is closed at the caudal end, although an apparently open anus is present.



FIG. 2.—*Opius fletcheri:* Mature egg. Length 0.66 mm.

[1] PEMBERTON, C. E., and WILLARD, H. F. A CONTRIBUTION TO THE BIOLOGY OF FRUIT-FLY PARASITES IN HAWAII. *In* Jour. Agr. Research, v. 15, no. 8, p. 419–465, 41 fig., pl. 32. 1918. Literature cited: p. 465.

This is the active stage of the larva, in which it is specially equipped with long, sharp mandibles for its struggle for survival over other larvæ of the same species, which it often finds in the same host individual. This struggle takes place immediately after hatching, and usually within four hours all but one of the larvæ of *Opius fletcheri* have been killed. Many cases have been observed where there were only one living and from two to eight dead parasite larvæ in the same host individual. Thus, having all the food material of its host available for itself, the surviving larva is able to proceed with its development to the adult stage.

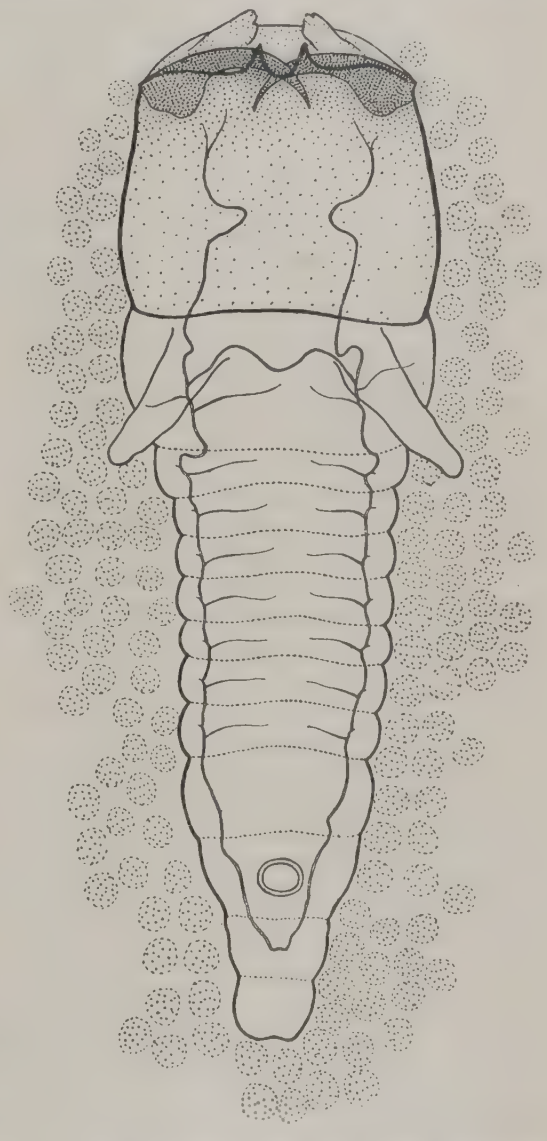


FIG. 3.—*Opius fletcheri:* Larva, first instar, ventral aspect, showing head characters and complete tracheal system, and the egg serosal cells. Length 0.88 mm.

The duration of this instar varies greatly and depends upon the development of the host. The larva never molts into the second instar until the parasitized host larva has formed its puparium. Several instances have been observed where larvæ of *Opius fletcheri* have developed to adults while other individuals, from eggs laid at the same time, still remained first-instar larvæ. The host larvæ of the former formed their puparia soon after they were parasitized, while those of the latter were still in the larval stage when examined. In all the experiments to prove this point the host was *Ceratitis capitata*, larvæ of which were feeding in the fruits of *Mimusops elengi*. These fruits become rather dry soon after falling from the tree, so that fruit-fly larvæ within them find difficulty in obtaining sufficient food for rapid development. This results in retarding pupation, sometimes for over three weeks beyond the normal period. On June 11 eggs of *O. fletcheri* were deposited into fruit-fly larvæ, which were examined with the following results: On June 18, 10 of these larvæ contained living first-instar larvæ of *O. fletcheri*, and 3, that had formed puparia, each contained a fourth-instar larva of *O. fletcheri*. On June 22, 3 more larvæ and 2 of the puparia of this lot were examined. Each of the larvæ contained a well-developed living larva of *O. fletcheri*

in the first instar, and each puparium contained a well-formed pupa of *O. fletcheri*. *C. capitata* larvæ into which *O. fletcheri* had deposited eggs on June 12 were examined on June 24. Each of 7 which were still in the larval stage contained a strong, living, first-instar larva of *O. fletcheri;* while 7 of the host larvæ, which had formed puparia, each contained a mature pupa of *O. fletcheri* about to emerge. Eggs that were deposited on June 13 produced, on June 27, 10 adult male *O. fletcheri*, and on June 28, 4 males and 2 females. On June 28, also, 2 of the host larvæ that had not yet pupated each contained a living first-instar larva of *O. fletcheri*. On June 14 eggs of *O. fletcheri* were deposited in fruit-fly larvæ. On June 27, 1 adult male *O. fletcheri* had developed from this lot, while 4 of the host larvæ, that had not formed puparia, each contained a living first-instar larva of *O. fletcheri*.



FIG. 4.—*Opius fletcheri:* Molted skin of first-instar larva, showing the absence of egg serosal cells. Length 0.8 mm.

These results indicate that the first instar of *Opius fletcheri* is controlled to a great extent by the development of its host, since it never molts into the second instar until the host has formed its puparium, and that the first instar may extend over a period of 10 to 12 days. When the host forms a puparium shortly after being parasitized, the first instar may be as short as 1½ days.

The second-instar larva (fig. 5) is very much without distinctive characters. The mandibles (fig. 6) are very small, soft, and indistinguishable even under high magnification, except upon occasions where the position and lighting are most favorable. They are 0.045 mm. in length and so far as can be seen serve no purpose. No tracheal system is present. None can be detected under the best of lighting and the highest of magnification. No part of the head or body is chitinized. The entire

body is very delicate and can be easily crushed beyond recognition with a very slight pressure on the coverglass. The digestive tract is simple and tubular and is closed caudally as in the first instar. In this stage the larva is sluggish in its movements, although it rapidly ingests a quantity of fat into its mid-intestine. Toward the latter part of this instar the mandibles of the third instar can be seen pushing at the bases of the mandibles.

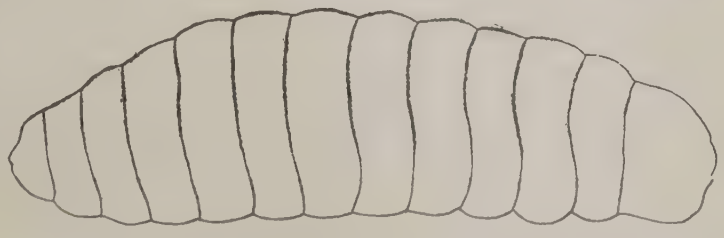


FIG. 5.—*Opius fletcheri:* New second-instar larva. Greatly enlarged.

The third instar, when first formed, is without a vestige of tracheæ. Tracheæ can be seen developing beneath the surface of the integument toward the latter part of this stage, but they are of the last instar and serve no purpose in the third. Few differences can be detected between this and the preceding instar, except an increase in size and a change in the shape of the mandibles. The third-instar larva measures 2.5 to 3 mm. in length. The mandibles (fig. 7) are somewhat more pointed and strong than those of the second instar; they bear no colored chitinization and measure 0.047 mm. in length. Toward the latter part of this instar the strong, chitinized mandibles of the last instar can be seen pushing at the bases of the mandibles.

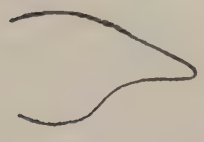


FIG. 6.—*Opius fletcheri:* Mandible of second-instar larva. Length 0.045 mm.

The mature, fourth-instar larva (fig. 8) averages 4 mm. in length and at its greatest width is about three-eighths as wide as long. When first molted into this instar it is 3 to 3.5 mm. long. The body is slightly curved, being concave ventrally, and, including the head, is composed of apparently 14 segments, although segment 14 is not clearly defined. A rather large, distinct spiracle is present on each side of segments 3, 5, 6, 7, 8, 9, 10, 11, and 12, counting the head as segment No. 1. These spiracles are joined on each side by a large lateral trunk extending nearly the length of the body. The trunks are connected near their caudal and cephalic extremities by a single, transverse, connecting trunk, these being the only connections between the two lateral systems. Branches from the lateral trunks extend dorsally and ventrally into each body segment, and prolongations of the lateral trunks extend into the head region. Portions of the body are covered by minute, strong, wide-based spines (fig. 9), which are closely set and abundant on the dorsal and lateral portions of body segments 2 and 3, counting the head as segment No. 1, and on the lateral areas of segments 4 to 12, inclusive. No spines occur on the head, on the articulation areas between the segments, or on the ventral portion of any segment of the body, and very few occur



FIG. 7.—*Opius fletcheri:* Mandible of third-instar larva. Length 0.04 7mm.

on the last segment. The only colored chitinized parts occur in the head, where a pair of strong, pointed mandibles (figs. 10, 11)—of which the distal half only is chitinized—and the tentorial structures are chitinized a yellowish brown color. Small maxillæ bearing minute papillæ are present, together with a well-defined labrum and suboval labium.

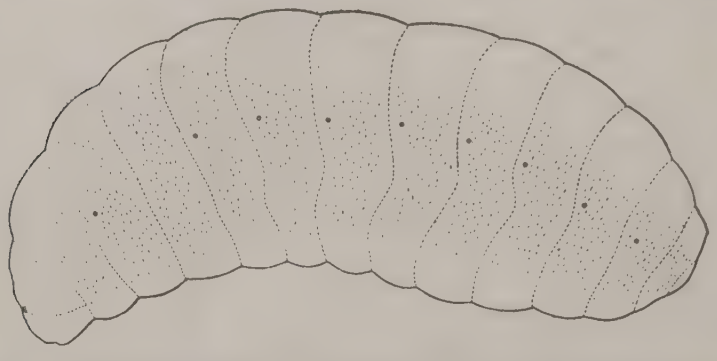


FIG. 8.—*Opius fletcheri:* Larva, fourth instar, lateral aspect, showing general outline and spiracles. Length 4 mm.

The most important changes that take place, then, during the larval development of *Opius fletcheri* occur in such a manner as to adapt it to the changing environment within its host. Larvæ of the first instar are very active and have long, sickle-like mandibles, which enable them to search out and destroy other parasite larvæ which occur in the same host individual. Second and third instar larvæ live in and feed upon the liquid or semiliquid medium contained in the host puparium. The mandibles, therefore, being useless, are small and inconspicuous, and there is no tracheal system whatever. In the fourth instar the liquid within the host puparium has been nearly all consumed, and the mature larva is found with fairly strong mandibles and a well-defined tracheal system connected with easily observed spiracles.

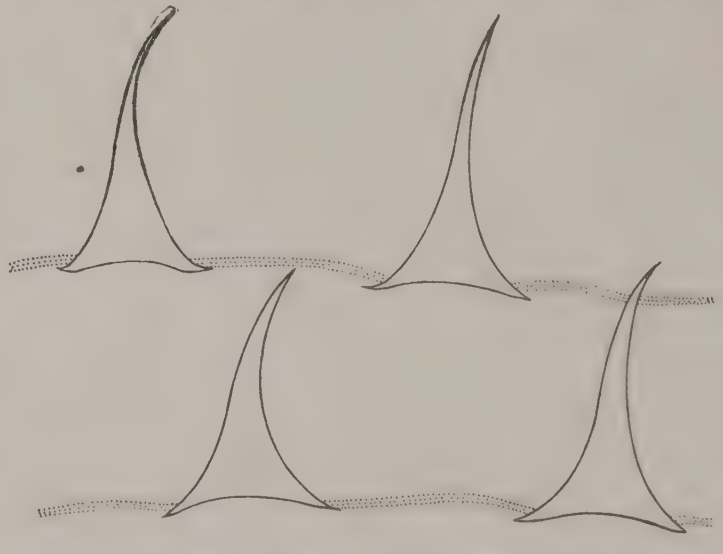


FIG. 9.—*Opius fletcheri:* Spines on body of mature larva. Length 0.013 mm.

Two species of opiine parasites of the Mediterranean fruit fly hibernate as mature larvæ for varying lengths of time during the cooler seasons of the year.[1] No hibernation of *Opius fletcheri* has been observed during any stage of its development, although thousands of parasitized puparia have been under observation. In September, 1918, 592 parasitized melon-fly pupæ were held in a refrigerator, where the temperature was constantly about 65° F., until two weeks after all adults had emerged. All unhatched puparia were then examined and no hibernating larvæ were found. One hundred and sixty

[1] PEMBERTON, C. E., and WILLARD, H. F. OP. CIT.
BACK, E. A., and PEMBERTON, C. E. THE MEDITERRANEAN FRUIT FLY IN HAWAII. U. S. Dept. Agr. Bul. 536, 119 p., 24 fig., 21 pl. 1918.

adults of *Opius fletcheri* emerged in the refrigerator, and each of the remaining 432 unhatched puparia contained a well-developed, dead pupa of *Opius fletcheri*. A control lot of 500 parasitized puparia that were held at the same time at normal temperatures, 75° to 85° F., produced 487 adult parasites and 13 dead pupæ of *Opius fletcheri*. Seventy-two and six-tenths per cent of the parasites developing in the refrigerator and 2.6 per cent of those developing at normal temperatures died while in the pupa stage. These data seem to indicate that it is difficult for *Opius fletcheri* to develop through the pupal stage at a temperature as low as 65° F. This mortality of pupæ, however, is not evident under field conditions. While records of parasitism of the melon fly, which was developing in cucurbits collected in the field at all seasons of the year, were being obtained, thousands of unhatched melon-fly puparia were opened. Although some of these records were secured when the temperature ranged from 60° to 70° F., less than 3 per cent mortality of *Opius fletcheri* pupæ was found. The cause of the high mortality of pupæ in the refrigerator has not been determined.

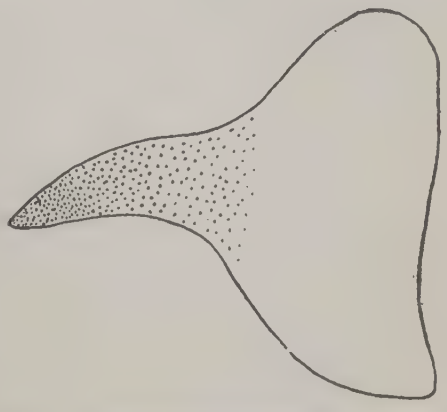


FIG. 10.—*Opius fletcheri:* Mandible of fourth-instar larva. Length 0.075 mm.

PUPA

In the process of transforming from the mature larva to the pupa (fig. 12) this insect passes through a prepupal state of from one to two days. The larva becomes motionless. The anterior portion of the body, which is to form the head and thorax of the pupa, becomes slightly contracted, so that it is somewhat smaller than the remainder of the body. The eyes can be seen, forming beneath the integument, as indistinct reddish brown spots; these become more distinct and darker in color until, just before the molt into the pupal stage, they can be plainly seen.



FIG. 11.—*Opius fletcheri:* Head of mature larva, dorso-cephalic aspect. Width 0.63 mm.

In the last larval molt the skin is split from the head backward and, by slight expansions and contractions of the body, it is pushed back over

the tip of the abdomen and finally comes to rest on the dorsal portion of the pupa. This exuvium often adheres to the antennæ of the male or the ovipositor of the female for a short time after the adult has emerged from the puparium of its host. The length of the pupa is 3.8 mm. When first formed it is pale white, excepting the eyes, which are a very dark reddish brown; but within a few hours it begins to acquire a yellowish tinge and continues to assume the colorations of the adult until ready to emerge.

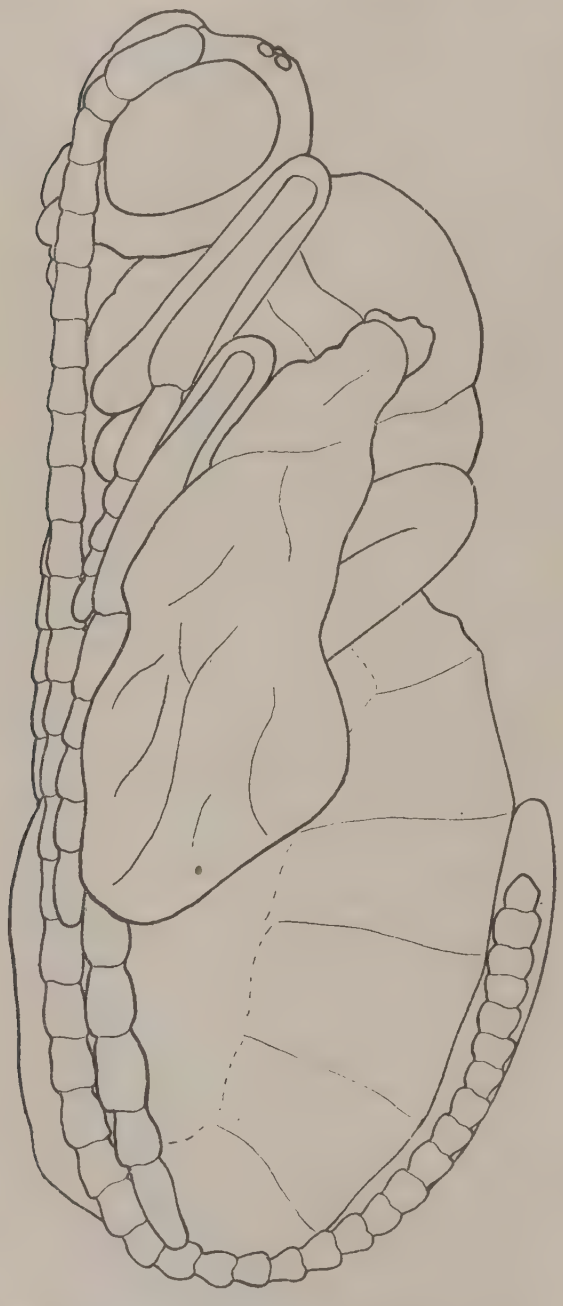


FIG. 12.—*Opius fletcheri:* Pupa, female. Length 3.8 mm.

The length of this stage varies from four to eight days, even though it is passed under the same temperature and other conditions. During the month of July, 1918, when the temperature ranged from 75° to 85° F., 90 parasitized puparia were under observation. Adults of *Opius fletcheri* emerged from these puparia from 80 to 200 hours after pupation. Emergence was taking place at frequent intervals between these two extremes but was most frequent between 130 and 150 hours after pupation. This would indicate that the length of the pupal stage in the majority of cases was about six days. Between 80 and 100 hours after pupation, 17 males emerged, but it was between 100 and 110 hours before the first two females emerged. The last male emerged after a period of from 170 to 180 hours, and the last two females emerged between 190 and 200 hours after pupation. The pupal stage of the male is usually about 24 hours shorter than that of the female.

ADULT

The following description of the adult by Silvestri is translated from the Italian:

Opius fletcheri, n. sp.

FEMALE.—Body ochreous yellow or testaceous, with the anterior part of tergites 2–6 of the abdomen brownish. Antennæ, except at the apex, where they are brownish, and legs, except the pale brown hind tarsi, of the same color as the body. Wings hyaline, with the nervures in great part brown. The stigma brown, except the middle part, which is yellowish white. Length of body 4.5 mm.; width of thorax 1.05 mm.; length of antennæ 6.5 mm.; of the wings 5 mm., width of same 2 mm.; length of ovipositor (the part protruding) 2 mm.

Head just a little wider than the thorax, about two-fifths wider than high, with eyes large, convex, nude, reaching below almost to the level of the margin of the clypeus. Face, excepting at the base of the antennæ, full, and subcarinate in the middle. Antennæ longer than the body, attenuate, composed of 42 to 48 segments, of which the scape is about five-eighths longer than the second segment.

Thorax.— esothoracic scutum with parapsidal grooves, indistinct, nude. The transverse prescutellar groove furnished with a series of about ten pits, not very deep. Metanotum lightly convex, and smooth in the middle for the greater part of its length, and carinate for a short space behind, pitted in the sides; propodium provided with a median longitudinal carina which divides behind, with a sublateral carina near the side, but within the stigmata, which are sufficiently large and round. The surface between the carinæ smooth. Mesopleura with the longitudinal groove crenulate.



FIG. 13.—*Opius fletcheri:* Adult female. Length 4.5 mm.

Anterior wings with the discoidal cell and the first cubital very large, subrectangular, longer than the second cubital, with the recurrent nervure long, arcuate as seen in the figure.

Abdomen suboval, with the first tergite lightly carinate at the side and lightly rugose in the middle. The rest smooth and furnished with a few long hairs, second suture rather distinct. Ovipositor, which is very sharp and straight, about as long as the abdomen.

MALE.—Similar to the female but a little smaller.

OBSERVATIONS.—This species of Opius is quite distinct from the numerous species I know from Palaeartic and Ethiopian faunas by the shape of the recurrent nervure, and by the length of the discoidal and first cubital cells.

HABITAT.—India. Prof. Fletcher obtained examples of this species from the pupæ of *Chaetodacus cucurbitae* Coquillett, the larvæ of which live in the fruits of *Momordica charantia* L.

The adult (fig. 13) liberates itself from the host puparium by gnawing a transverse slit near the end and by pushing with its head until the entire end of the puparium breaks off, allowing it to emerge. Immediately after emergence the meconium is discharged. This meconium is an ovoid, hard pellet, consisting of all the waste material which has collected in the digestive tract during the larval stage. No waste material is voided before this time, although many braconids discharge it just prior to pupation.

Copulation may occur frequently, and at any time, from immediately after emergence to the death of the adult. Two newly emerged females were put into a glass tube with one male that had just emerged, and the male successfully copulated with both females within 10 minutes. Nine females that emerged May 18 to 20 were put into a tube with males, where several instances of successful mating were observed. On July 1, when these females were 6 weeks old, they were put into a glass tube with 30 newly emerged and vigorous males. Within 45 minutes 12 successful matings were observed, and one of the females mated four times within 15 minutes. In all of these instances the females made no great effort to escape from the males. The period of coitus lasts from ½ to 2 minutes, although in the majority of instances it is less than 1 minute. In six of eight cases under observation the duration was between 30 and 45 seconds, while in the other two cases it was extended to 1½ and 2 minutes, respectively. As far as it has been possible to observe, all of the sex attraction is produced by the male. When within about 2 inches of the female, the male becomes greatly excited and while slowly approaching her, and during coitus, vibrates the wings vigorously and spasmodically. No strong, sweet odor, such as is emitted by the males of the fruit-fly parasites *Opius humilis* Silvestri and *Diachasma tryoni* Cameron,[1] has been detected during work with this species.

Opius fletcheri is capable of parthenogenetic reproduction, and the absence of mating does not influence oviposition. Large numbers of adults, all of which were males, have been reared from unmated females. The fact that mated females will produce a considerably larger percentage of females than males is of much interest. Eight females that were observed mating within two hours after emergence were put into individual glass tubes, where host larvæ were available at all times. From these females 39 males and 72 females were reared, giving 35.1 per cent males and 64.9 per cent females. Under field conditions about 10 per cent more females than males are produced. While records of parasitism of the melon fly developing in cucumbers collected in the field during 1918 and 1919 were being secured, 7,746 adult *O. fletcheri* were reared. Of this number 4,273, or 55.2 per cent, were females, and 3,473, or 44.8 per cent, were males. Many species of opiine parasites consistently produce more males than females. For example, the parasites of the Mediterra-

[1] PEMBERTON, C. E., and WILLARD, H. F. OP. CIT.

nean fruit fly, *D. tryoni* and *O. humilis*, that were reared from material collected in the field, produced 37.6 per cent and 43.5 per cent females, respectively. Since the females are responsible for all the parasitism of the host, the ability of *O. fletcheri* to produce so many more females than males greatly enhances its value as an enemy of the melon fly.

The longevity of the adult depends largely upon the conditions under which it lives and may extend from a few days to 16 weeks. When confined without food it will not live much over 5 days. Of 6 males and 17 females that were confined in a glass tube without food, 3 females died before they were 3 days old, and 3 more lived to be a few hours over 5 days old, but the majority of both males and females died between the ages of 3½ and 4 days. The life of females that have had continual access to host larvæ is much shorter than that of those which have had no opportunity to oviposit; and the life of males is considerably shorter than that of the females. Of 9 females that were allowed to oviposit at will, 2 died at the end of 2 weeks, 2 at the end of 8 weeks, and the other 5 lived 3, 5½, 6, 6½, and 7 weeks, respectively. With no opportunity to oviposit, 85 females, together with 43 males, were confined in a glass tube and kept in partial darkness, with daily feedings of a mixture of one-fourth honey and three-fourths water. Three of these females lived to be 16 weeks old, 33 of the males died between the ages of 6 and 8 weeks, while 1 male lived to be 11 weeks old. The majority of the females died between the ages of 11 and 13 weeks, while 15 lived a little beyond this period.

OVIPOSITION

Oviposition takes place in only the larva of the host and may occur at any time after the larva is one-half grown; but it is most frequent in well-developed larvæ. Observations of the female, just prior to oviposition, indicate that she locates the host larva beneath the skin of the containing fruit by a sense of touch. She walks rapidly over the surface of an infested fruit, stopping at frequent intervals, evidently endeavoring to detect vibrations caused by a feeding host larva. While searching for the host, and during the act of oviposition, the female often vibrates her wings rapidly and spasmodically, although this does not always happen. When a favorable spot is found, she elevates her abdomen and pierces the skin and pulp of the fruit with her ovipositor, raising and lowering it until the host is located. She then inserts the ovipositor into the larva and deposits an egg just beneath the skin. Then she withdraws the ovipositor from the fruit and usually begins to search for another larva; but occasionally, after a short rest, she will oviposit again in the same one. The female is unable to discern between parasitized and unparasitized larvæ.

Although mating may occur immediately after emergence, oviposition does not begin until 2 days later and, in the majority of cases, 3 to 5 days after emergence. Eight fertile females were given constantly

available host larvæ from the time of emergence. Two of these began ovipositing in 2 days, one in 3, three in 5, and two in 7 and 9 days, respectively. None of these females oviposited after they were 30 days old, excepting one, which deposited one egg at the age of 33 days. The majority of eggs are deposited within the first 3 weeks after oviposition begins. As noted before, females that have had daily opportunity to oviposit do not live so long as those that have had no opportunity; but they frequently live from 4 to 5 weeks after oviposition has ceased.

IMPORTANCE AS A PARASITE

Opius fletcheri, in the three years since its introduction into the Hawaiian Islands, has become firmly established on all the large islands of the group. While this parasite alone will never exercise a complete control over the melon fly in Hawaii, it has already proved of much value by decreasing the numbers of this pest considerably. Good examples of the most abundant melon-fly host plants are cucumber, squash, pumpkin, and watermelon. The fruits of these plants are large and fleshy, and melon-fly larvæ that develop in them feed so far from the surface that a larval parasite, such as *O. fletcheri*, that oviposits entirely from the outside, finds it impossible to parasitize enough of the larvæ to exert a control over the pest.

Table I gives data showing the extent of parasitism by *Opius fletcheri* of melon-fly larvæ developing in cucumbers collected in and about Honolulu during the last eight months of 1918 and the first eight months of 1919.

TABLE I.—*Percentage of parasitism by Opius fletcheri of larvæ of Bactrocera cucurbitae in cucumbers*

Month of collection.	Number of larvæ emerging during first two to four days.		Percentage of parasitism.	
	1918	1919	1918	1919
January	.....	1,031	.....	2.9
February	.....	539	.....	14.5
March	.....	6,442	.....	9.0
April	.....	3,192	.....	1.3
May	1,014	1,481	5.9	2.2
June	2,719	1,318	10.0	6.4
July	2,052	5,255	21.9	10.6
August	431	19,321	21.8	7.3
September	3,594	.....	29.8	.....
October	2,516	.....	16.6	.....
November	8,282	.....	22.1	.....
December	4,319	.....	7.3	.....

The highest percentage of parasitism existed in September, 1918, when 1,070 out of 3,594 melon-fly larvæ under observation were parasitized. This shows a parasitism of 29.8 per cent, while the parasitism

from all cucumbers collected during 1918 was 18.1 per cent. Parasitism from larvæ developing in cucumbers collected in the first eight months of 1919 amounted to 7.3 per cent. These records were secured from only those larvæ that emerged from the cucumbers the first two to four days after collection. Larvæ emerging after this time would not give a true representation of parasitism under field conditions, because at the time they were collected they were comparatively small and had been subject to parasitism only a short time. These cucumbers were specially selected by the collector as being the most heavily infested ones in the fields. Considering the fleshy nature of cucumbers and the fact that those from which these data were obtained were from 4 to 10 inches long, it is remarkable that *Opius fletcheri* is able to destroy such a high percentage of the melon-fly larvæ developing in them.

Considerable effort has been made to establish a series of records a comparison of which would show the amount of infestation by the melon fly from time to time and which would determine the extent of control exerted by *Opius fletcheri*. Infestation records of the Mediterranean fruit fly have been secured by recording the average number of larvæ per fruit, this average being obtained from a large number of fruits of the same species. The great variation in size of cucumbers made this method impracticable, and the following method was used: All cucumbers that were collected for records of parasitism were weighed and then held until all the melon-fly larvæ had emerged. Accurate records of these larvæ were kept, and at the end of December, 1918, and of August, 1919, the average number of larvæ per pound of host fruit was obtained. From July to December, 1918, inclusive, 200 pounds of cucumbers were collected, which contained 47,888 melon-fly larvæ, or an average of 239.4 per pound. From 337 pounds of cucumbers, collected during the first eight months of 1919, 57,921 melon-fly larvæ were secured, giving an average of 172 larvæ per pound. These averages indicate that the melon-fly infestation of cucumbers in and about Honolulu was approximately 28 per cent less during the period from January 1 to August 31, 1919, than it was between July 1 and December 31, 1918.

It appears from observations of melon-fly infestation in Hawaii made during the past several years that this decrease in the numbers of the melon fly is due to a great extent to the activities of *Opius fletcheri*. Before this parasite was introduced into Hawaii in 1916 it was almost impossible to find a cucumber in the Honolulu markets that did not show more or less evidence of attack by the melon fly. From observations made by them in 1915 and 1916, Back and Pemberton state [1] that one rarely sees cucumbers offered for sale in the Honolulu markets that do not show some evidence of attack, even when carefully selected, and that during midwinter 150 out of 152 cucumbers ready for market

[1] BACK, E. A., and PEMBERTON, C. E. THE MELON FLY IN HAWAII, U. S. Dept. Agr. Bul. 491, 64 p., 24 pl., 10 fig. 1917. Bibliography, p. 57–64.

at Moiliili were found variously infested. They state also that the ordinary cucumber, when very young, is the most resistant to melon-fly attack of all the cucurbits cultivated in Hawaii, but that inasmuch as the fly has been permitted to increase unchecked since its introduction it has become so abundant that slight differences in inherent resistance to attack are not evident among host fruits growing in the field. The condition of cucumbers offered for sale in Honolulu during the first eight months of 1919 indicates that *O. fletcheri*, while not being able completely to control the melon fly on the island of Oahu, has been able to reduce its numbers to such an extent that the infestation of cucumbers has been greatly decreased. During this period there have been good quantities of this vegetable on the market at all times, a very small portion of which has shown evidences of melon-fly attack. The writer has observed on several occasions at different plantations wagon loads of cucumbers that had been selected for market, among which it was difficult to find any great number that had been attacked. While collecting cucumbers during the past year from the different gardens for parasitism records, it has often been difficult to get a sufficient quantity of well-infested fruits. These observations, as compared with those made previous to the establishment of *O. fletcheri*, would lead to the conclusion that this parasite has already become of much value, even while attacking its host in the larger cucurbits.

The ability of *Opius fletcheri* to reach and parasitize the majority of host larvæ developing in the smaller fruits is clearly shown by data collected during the past five years in the Kona district of the island of Hawaii. In this district it comes nearer to controlling the melon fly completely than in any other locality that has been observed. This great degree of control is without doubt due to the great abundance of the wild Chinese cucumber (*Momordica* sp.). The fruits of this plant are small, about ½ to 1½ inches in diameter by 1 to 2 inches long. The following observations give a good conception of their susceptibility to melon-fly attack and of the ability of *Opius fletcheri* to decrease their infestation greatly by parasitizing a large percentage of the larvæ developing in them.

From observations made in this district, Back and Pemberton state[1] that—

> From Momordica vines covering a patch of pasture land 6 feet square, 331 fruits were gathered during November, 1914, of which only 12 had not been infested. These fruits, which were of all sizes up to 1½ inches in diameter, averaged between three and four punctures per fruit, with a maximum of 15 punctures on the more exposed fruits. From 7 feet of stone wall 442 fruits were gathered, and of these 193 were so badly affected that they had dried up without developing seeds, and only 11 were not affected. From 250 fruits placed over sand 1,586 larvæ, or an average of 6.5 larvæ per fruit, were reared.

[1] Back, E. A., and Pemberton, C. E. The melon fly in Hawaii. U. S. Dept. Agr. Bul. 491, 64 p., 24 pl., 10 fig. 1917. (See p. 17–18.)

A careful examination of 442 fruits of Momordica, collected at random over an area of ¼ square mile in the Kona district, made by C. E. Pemberton on May 8, 1916, gave the following results: 194 were not infested, and the 248 that were contained a total of 559 eggs and 1,222 larvæ of the melon fly. This is an average infestation per fruit for the 442 fruits of 4 flies either in the egg or larval stage.

The first adults of *Opius fletcheri* were liberated in this district in the summer of 1916. Data secured by C. E. Pemberton during the latter part of April and the first part of May, 1918, showed that it had become widely established, was parasitizing a very high percentage of the melon fly developing in Momordica, and that it had so greatly reduced the number of flies that cultivated cucurbits were being raised with little or no infestation. Out of 1,706 Momordicas collected by him on April 25 and 26, 1918, 347 fly larvæ emerged the first two days after collection, of which 299, or 86.2 per cent, produced parasites. On April 28 and 29, 700 Momordicas were collected, from which 226 melon-fly larvæ emerged during the first two days. Of these 226 larvæ 219, or 96.9 per cent, produced parasites. From these two lots 103 larvæ emerged after the first two days, making a total of 676 larvæ developing in 2,406 fruits. This is an average of less than 0.3 larva per fruit, as compared with an infestation of from 4 to 6.5 larvæ per fruit before the liberation of *O. fletcheri*.

Further observations made at the same time of 1,706 ripe Momordicas collected in the same locality showed that only 36 of this number contained either eggs or larvæ of the fly. Thirty ripe fruits of the same plant, collected at Honaunau, about 12 miles from Kealakekua, showed no infestation whatever. On May 10, 1918, 400 cucumbers, both large and small, 28 young watermelons, 20 young muskmelons, and 21 young pumpkins were carefully examined in a garden in Kealakekua. This garden was bounded on one side by a coffee plantation and on the other three sides by pasture land that was overrun with heavily-fruiting vines of wild Momordica. Only one cucumber was found that had been punctured by the melon fly. None of the other vegetables or melons that were examined had puncture scars, either new or old, and none of the blossoms of any of the plants were stung.

In June, 1919, this same low degree of infestation still existed in this district. From 890 Momordicas collected at that time the average infestation was less than 0.2 larva per fruit. In several gardens less than 3 per cent of the cucumbers and melons that were examined showed evidences of attack, and none of the blossoms were found that had been stung.

When the vines of wild Momordica are abundant on pasture lands, their ability to cover and kill large patches of grass has caused them to be considered a pest, and consequently they have not been allowed to

become abundant in many localities in Hawaii. When Momordica is abundant and *Opius fletcheri* is present, it has proved of considerable value as a trap plant for the melon fly. Infestation records made before the parasite was liberated show that Momordica is much favored as a host by the melon fly, while subsequent records of parasitism show that its size and texture permit the parasite to kill about 90 per cent of the larvæ developing in its fruits. Whether or not it would be of advantage to plant these vines around vegetable gardens as a catch plant is a problem open to further investigation.

Opius fletcheri, besides becoming firmly established on all the larger islands of the group, has shown itself capable of reducing the number of melon flies by at least 25 per cent, even when the host larvæ are developing in fruits the size and nature of which make parasitism difficult. In a location where the fruits and conditions are most favorable to its reproduction it has reduced the flies so greatly that they have almost ceased to be a pest. While *O. fletcheri* is far from being able to control the melon fly in Hawaii completely, the benefits derived from its activities since its establishment there have been sufficient to warrant the efforts connected with its introduction.

TAMARIND POD-BORER, SITOPHILUS LINEARIS (HERBST)[1]

By RICHARD T. COTTON, *Scientific Assistant, Stored-Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

The literature of North American entomology contains occasional reference to the curculionid beetle, *Sitophilus linearis* (Herbst), but nothing definite has been published regarding the biology of this interesting weevil or the extent of its distribution in the United States.

HISTORY AND DISTRIBUTION

The tamarind pod-borer was described in 1797 by Herbst under the name of *Rhynchophorus linearis*. The specimens described were obtained from the West Indies, where the weevil had been introduced with its food plant, the tamarind. It undoubtedly is native to India but has now spread to all places where the tamarind is grown. In 1815 it was described by Thunberg as the variety *striata*, and again in 1834 by Christy under the name of *Calandra tamarindi*, and finally in 1837 by Dejean under the specific name of *frugilega*. All of these later names have since been reduced to synonymy.

In 1892 Casey[2] noted the occurrence of *Sitophilus linearis* in North America, but in 1895 Chittenden[3] stated that in his opinion *S. linearis* should not be inserted in our faunal list until it could be ascertained that the species actually bred in some plant within our faunal limits. Up to the present time all records of its occurrence in the United States refer to occasional specimens picked up in the southern Atlantic and Gulf States which had undoubtedly been imported in shipments of tamarind pods. The writer has found it to be exceedingly abundant in southern Florida where the tamarind is now grown; therefore there is no longer any doubt that it is well established within our faunal limits.

In 1916 A. H. Ritchie[4] recorded this species as causing considerable damage to the pods of the tamarind in Jamaica, and T. B. Fletcher[5] has recorded similar damage in India.

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[1] The writer was enabled to make a study of this species through the courtesy of the Federal Horticultural Board, whose representative, Mr. O. K. Courtney, intercepted at the port of New Orleans a shipment of infested tamarind pods from Guatemala, which was forwarded for study to the division of Stored-Product Insect Investigations of the Bureau of Entomology. The writer wishes to acknowledge his indebtedness to Dr. Adam G. Böving, of the Bureau of Entomology, for his valuable aid and advice in the study of the larval characters of this weevil.

[2] CASEY, Thos. L. COLEOPTEROLOGICAL NOTICES IV. *In* Ann. N. Y. Acad. Sci., v. 6, 1891–92, p. 359–712. 1892. [*Calandra linearis*, p. 686.]

[3] CHITTENDEN, F. H. ON THE DISTRIBUTION OF CERTAIN IMPORTED BEETLES. *In* Insect Life, v. 7, no. 4, p. 326–332. 1895.

[4] RITCHIE, Archibald H. REPORT OF ENTOMOLOGIST FOR YEAR 1915–1916. *In* Ann. Rpt. Dept. Agr. Jamaica [1915] 16, p. 31–34. 1916.

[5] FLETCHER, T. Bainbrigge. ONE HUNDRED NOTES ON INDIAN INSECTS. *In* Agr. Research Inst. Pusa Bul. 59 39 p., 20 fig. 1916. Weevils in tamarind fruits, p. 10.

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This weevil is now known to occur in the United States, India, Brazil, Mexico, Ecuador, Jamaica, Montserrat, St. Bartholomew, Cuba, and Costa Rica. It occurs, undoubtedly, wherever the tamarind is grown.

NATURE OF INJURY

The injury is confined entirely to the seed pods of the tamarind. The adult weevils feed little, but the larvæ or grubs bore in the seeds or beans and reduce them to powder. The entire crop is frequently completely destroyed unless promptly harvested and protected.

For those not familiar with the tamarind a few descriptive and historical notes are here inserted.

The tamarind, *Tamarindus indicus*, although attributed to India, is positively asserted to be indigenous to Africa and Australia. It was introduced into the West Indies by the Spaniards soon after the discovery of those islands, and was naturalized at an early date in Brazil, Ecuador, Mexico, and other parts of the tropical world. A few trees have been introduced into the United States in Florida and California. Although a tropical plant it does well in southern Florida.

The seeds are borne in large pods and are embedded in a sweet, sticky, reddish pulp. This pulp has mild laxative properties and is found on the market usually mixed with sugar or syrup. In tropical countries the pulp is used extensively for the preparation of a cooling beverage and as a flavoring for ice cream. In European countries it is said that the pods and seeds when roasted are considered a delicacy. The bark, seeds, and leaves are used to a limited extent by natives of the Tropics as therapeutic agents.

The wood is heavy and hard and is used for making furniture on account of its fine grain and color. It is used also in making tools, axles, wagon wheels, and similar articles.

LIFE HISTORY AND BIOLOGY

Since the tamarind grows only in tropical or subtropical climates, the activities of the weevil are not stopped by winter. It breeds throughout the year. In Florida the seeds of the tamarind usually mature in May, but a few may be found maturing in almost all months of the year, thus providing a more or less continuous food supply for the weevils. As the pods mature they quickly become infested.

The adult weevils enter the tough-shelled pods through the stem end. The swaying of the pods in the wind causes small breakages in the pod rind to occur at the juncture of the stem, and through these breaks the weevils find an easy entry. The female weevils bore directly through the pulpy covering and into the tough seeds. In the seeds they excavate a cylindrical cavity about 3 mm. deep and 1.5 mm. in diameter. If the shell of the pod is broken away the weevils may be seen at work, the top

of the abdomen alone showing above the surface of the pulpy covering, the rest of the body being concealed within the cavity. This cavity is usually completed in from two to three days. The individual egg cavities are then bored in the seed all around the interior of this larger cavity, an egg being deposited as soon as a hole is finished. The eggs are all placed as close together as possible, so that the interior of the large cavity has the appearance of being lined with rows of egg-caps. From 12 to 50 eggs are laid in one group, the time taken for the completion of the group varying from one to two weeks, according to the number of eggs laid. By the time the last egg is laid the first eggs have hatched and the grubs have become half grown. This habit of the female weevil of grouping a number of eggs together in one seed exhibits an interesting difference from the egg-laying habits of the grain weevils belonging to this genus. One would naturally conclude that it was developed to save energy, since it would be no mean undertaking to bore through the pulpy covering and the tough seed coat for each individual egg.

The operation of excavating the egg cavities is accomplished by a combined up and down and rotary motion of the proboscis, effected by turning the head from side to side while the thorax is oscillated back and forth. As soon as an individual egg cavity is completed and the sides are smoothed to the satisfaction of the weevil the proboscis is withdrawn. The weevil then reverses its position and, inserting its ovipositor into the cavity, deposits an egg, sealing it in with a plug of opaque, yellowish material resembling faecal matter. In a few days this plug turns to a dark yellowish brown.

It is interesting to note that, so far as observations go, the female weevil does not leave the egg cavity from the time it is started until the last egg has been laid. She works day and night until the operation has been accomplished unless disturbed by outside agencies. Whenever she rests it is without leaving her position in the cavity. As soon as one group has been finished the weevil immediately seeks out another location and begins operations again. For sheer industry and continuous application to the object of perpetuating its kind this weevil would be hard to surpass.

The eggs hatch at the end of three days. Previous to hatching the larva may be distinctly seen through the thin outer shell of the egg. This shell or skin is very flexible and undulates with the movements of the young grub. It becomes somewhat wrinkled and finally breaks at the bottom, allowing the grub to escape. The young larvæ begin at once to feed and bore through the seed, their burrows radiating from the large cavity to all parts of the seed, and usually ending near the shell of the seed, through which, however, they never break.

As in other species of this genus, there are four larval instars, although previous writers have erroneously attributed but three larval instars to

the grain weevils of this genus. The lengths of the various stages are regular and are given in Tables I and II.

TABLE I.—*Life history data of the tamarind pod-borer* [1]

Weevil No.	Egg laid.	Hatched.	First molt.	Second molt.	Third molt.	Prepupa.	Pupa.	Adult.
1	June 19	June 22	June 24	June 26	June 28	July 5	July 6	July 13
2	23	26	29	July 1	July 3	11	12	19
3	25	28	30	2	4	12	13	20
4	26	29	July 1	3	5	13	14	21
5	29	July 2	4	6	8	14	15	21
6	July 2	5	7	9	11	16	17	24
7	2	5	7	9	11	17	18	25
8	4	7	9	11	13	21	22	28
9	4	7	9	11	13	21	22	29
10	11	14	16	18	20	24	25	31

[1] Data included in tables were secured at Orlando, Fla., during June and July, 1919. The mean temperatures for period were as follows: June, average mean temperature 79.4° F., high mean 90.5°, low mean 68.3°; July, average mean temperature 81.4°, high mean 92.4°, low mean 70.3°.

TABLE II.—*Length of stages of the tamarind pod-borer*

Weevil No.	Egg.	First larval stage.	Second larval stage.	Third larval stage.	Fourth larval stage.	Prepupal stage.	Pupal stage.
	Days.	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*
1	3	2	2	2	7	1	7
2	3	3	2	2	8	1	7
3	3	2	2	2	8	1	7
4	3	2	2	2	8	1	7
5	3	2	2	2	6	1	6
6	3	2	2	2	5	1	7
7	3	2	2	2	6	1	7
8	3	2	2	2	8	1	6
9	3	2	2	2	8	1	7
10	3	2	2	2	4	1	6

The pearly white larvæ, when full grown, construct pupal cells within the seed by lining the cavities at the end of their larval burrows with a mixture of frass and borings cemented together with a secretion that gives it when dry the appearance and consistency of a dark brown shellac.

As shown in Table II the larval stage usually requires from 12 to 14 days. After a prepupal stage of about 1 day the pupal form is assumed, and 7 days later the adult is formed. The adult does not immediately leave the seed but remains within to harden and feed for a few days. It then makes its way to the original cavity made by the mother weevil when laying her eggs and emerges, rarely if ever forcing its way through the shell at any other point.

After the adults have all emerged little is left of the seed but the empty shell and a mass of powder.

PREOVIPOSITION PERIOD

Copulation takes place soon after emergence, and the females deposit their first eggs in from 7 to 10 days after attaining adult form. Copulation is frequent and often takes place while the female is at work on the egg cavity.

OVIPOSITION PERIOD

The longest oviposition period recorded lasted for 84 days, and during this time 183 eggs were deposited. Toward the latter part of this period fewer eggs were laid than at first, the female becoming more and more feeble and exhausted. Three weeks after the last egg was laid the female died. The male died a few days later.

Other female weevils in captivity deposited from 126 to 165 eggs. It seems probable that under natural conditions with an abundant supply of fresh seed the oviposition period would be longer and the number of eggs deposited would be correspondingly larger.

HABITS OF ADULT

The males are, as a rule, slightly more abundant than the females. Of 488 bred specimens, 258, or about 53 per cent, were males. The males apparently feed but seldom, spending their time in constant attendance on the working females or in fighting among themselves for the females. They are of a very combative nature, and it is not uncommon to see two and sometimes three males fighting together for hours at a time with apparently great ferociousness. As they have no efficient or deadly weapons, however, little damage is done; and long before a decision is reached another male has assumed the care of the female, who, intent only on her work, is oblivious to the struggles of the aspiring males. The males are readily distinguished from the females by their shorter, thicker beaks. The beak of the male is considerably broader at the base than that of the female. The adults in captivity have fed on acorns, sweet potatoes, and various fruits. Normally, however, they do not attack anything but the tamarind seeds.

PARASITES

No parasites have been reared from any of the stages of *Sitophilus linearis*. Larval and pupal stages in the laboratory were attacked and killed by a predacious mite, *Pediculoides ventricosus* Newport. It seems very doubtful, however, that this mite would be able to penetrate to the larval burrows under field conditions.

DESCRIPTION OF IMMATURE STAGES

EGG

The egg is opaque, white, shining, ovoid to pear-shaped, rounded at the bottom; the top is slightly flattened and narrower, fitting into a plug or cap that cements it into place. The shell of the egg is very delicate and flexible, conforming to the shape of the egg cavity. Its length is 0.60 to 0.64 mm., the width 0.31 to 0.35 mm.

MATURE LARVA

The mature larva measures from 2.5 to 3.5 mm. in length and is pearly white in color. It is a footless, fleshy grub, very thick-bodied, the ventral outline being approximately straight while the dorsal outline is almost semicircular. The head is light brown in color, the anterior margin and mandibles are much darker, the head is longer than broad and somewhat wedge-shaped, and the sides are broadly rounded from middle to apex. The apex is slightly angular. The sides are nearly straight from the middle to the anterior angles, and the lateral area has an oblique, longitudinal, lighter stripe or area. The epicranial and frontal sutures are distinct and light in color; there are also two oblique, longitudinal, light stripes rising from the frontal sutures and coalescing with the epicranial suture near the apex. The frons is subtriangular with a distinct dark median line from the posterior angle to the middle, indicating a carina. The sutural margins are irregular or sinuate. The frons is provided with five pairs of large setæ, and each sutural margin bears a large seta. Each epicranial lobe bears the following setæ: One close to the posterior angle of frons and located within the oblique, longitudinal stripe rising from the frontal suture; one very small seta posterior to this and near occiput, two anterior to it on disk of epicranium; two opposite middle of frons; one opposite middle of mandible; one opposite hypostomal angle of mandible; and one on hypostoma near base of mandible. The epistoma is represented by the thickened anterior margin of the front. It is distinctly darker in color, with the anterior margin declivous and slightly curving and the lateral angles slightly produced and elevated where they support the dorsal articulation of the mandibles. The pleurostoma is represented by the somewhat darker declivous area surrounding the mandibular foramen. The mandibles are stout, triangular, with the apex produced into an acute apical tooth. The inner edge toward the apex is provided with a subapical tooth and a small medial tooth, no molar parts present. The dorsal area of the mandible is provided with a pair of bristles set apart. The eye is represented by a well-defined black spot beneath the exoskeleton.

The clypeus is attached in front of the frons and is broadly transverse. It is broad at the base, the sides narrowing toward the apical angles, and is slightly longer and broader than the labrum. It bears on the epistomal margin two fine setæ on each side. The labrum is distinctly broader than long, with two lateral and a larger median lobe. It is provided with six large setæ behind the middle, two marginal, short, thickened setæ on each of the lateral lobes, and six similar marginal setæ on the median lobe.

The cardo is present and distinct in the maxilla; the stipes is not divided into stipes proper, subgalea, and palpifer but is one continuous piece, with the anterior inner angle produced into a single setose lobe.

The palpus is 2-jointed and bears a single seta near the apex of the first segment. There are three other setæ found on the maxilla, two located on the vaginant membrane between the palpus and palpifer, and one stouter and longer seta midway between the palpus and cardo. There is no articulating maxillary area between the maxilla and the mental-submental region.

The submentum and mentum are fused and are represented by a broad lobe bearing three pairs of stout setæ. The stipes labii are posteriorly enforced by a median, trangular chitinization; the anterior median section is produced anteriorly between the palpi into a small lobe-like ligula which is fused with the lingua. Each stipes labii bears a single seta. The short, conical, 2-jointed palpi are situated on the anterior angles of the stipites. The ligula bears four small setæ.

The prothorax is dorsally not divided; but two areas, the praescutal and scutoscutellar areas, are roughly indicated by rows of setæ. The mesothoracic and metathoracic segments are above divided into two distinct areas, the anterior of which represents the praescutum and the posterior the scuto-scutellum and alar area. The thoracic spiracle is located on a lobe pushed into the prothorax from the epipleurum of the mesothorax. It is bifore, elongate, larger than the abdominal spiracles, and placed with the finger-like air tubes pointing dorsad. The metathoracic spiracle is rudimentary.

There are 10 abdominal segments, the first 7 similar, the last 3 smaller and reduced. Each of the abdominal segments 1 to 8 is supplied with a spiracle, that of the eighth being located more dorsally than the rest. Each tergum is divided above into two distinct areas. The first contains praescutal and scutal elements; the second represents the scutellum. Below these two areas and adjacent to the epipleurum is the alar area. The abdominal spiracles are placed anteriorly and in a little separate corner piece, probably of the alar area.

Below a very indistinct and abrupt dorso-lateral suture and above a well-defined ventro-lateral suture is a large, not subdivided epipleurum. The abdominal epipleura are located considerably higher than the thoracic, and the ventro-lateral suture makes an S-shaped line between metathorax and first abdominal segment. Below the ventro-lateral suture is the hypopleurum subdivided into three lobes, one right under the other. Below the hypopleurum is the coxal lobe, and below that is the sternum, consisting of the eusternum and a posterior triangular area representing the parasternum or the parasternum fused with the sternellum.

The setæ on the abdominal segments are arranged as follows: One on the praescutum, a long and two shorter ones on the scutellum, two on the alar area located just above the spiracle, two on the epipleurum, one on the middle lobe of the hypopleurum, one on the coxal lobe, and three on the eusternum. One of the hairs on the scutellum is sometimes missing on the last few abdominal segments.

LARVAL INSTARS

First-instar larva 0.53 to 0.60 mm. long, 0.37 to 0.43 mm. wide; pearly white; head about 0.25 mm. wide, 0.26 long.

Second-instar larva 0.65 to 0.80 mm. long, 0.5 to 0.65 mm. wide; head 0.32 mm. wide, 0.36 mm. long.

Third-instar larva 0.75 to 1.3 mm. long, 0.6 to 1 mm. wide; head 0.42 to 0.45 mm. wide, about 0.52 mm. long.

Fourth-instar larva 1.5 to 3.5 mm. long, 1 to 2.5 mm. wide; head about 0.57 mm. wide, about 0.80 mm. long.

PUPA

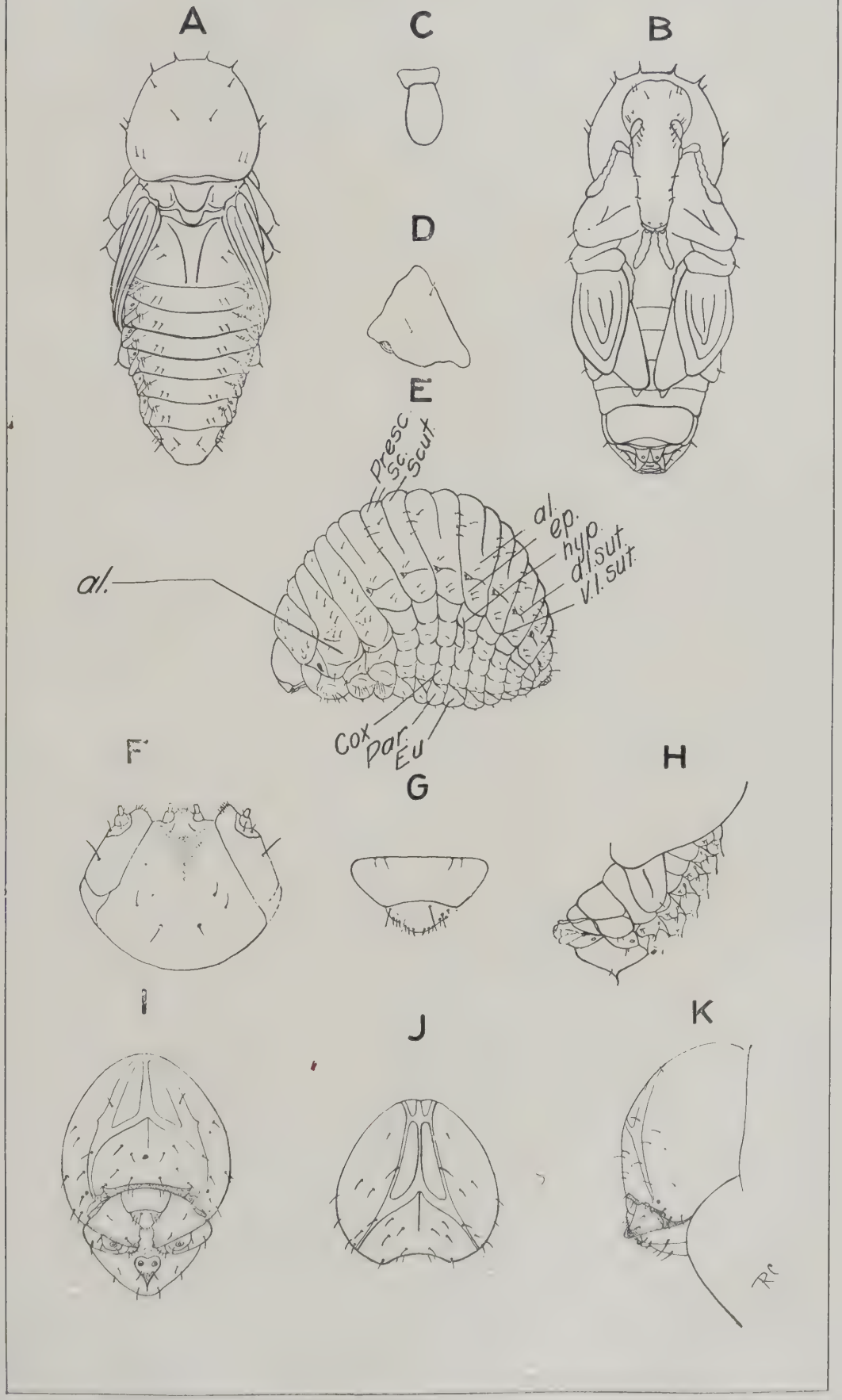
The pupa is uniformly white when first transformed, 3.5 to 4.25 mm. long, and about 1.65 mm. wide. The tips of the wing pads attain the fifth abdominal segment; the tips of metathoracic tarsi extend beyond the tips of the inner wings. The head is oval, the beak elongate and slender. The head has two prominent spines towards the vertex, a group of two spines and two spinules on each side above the eyes, two pairs of small spines near the anterior margin, and a small one on each side of the front between the eyes. There are three pairs of spines on the beak between the frontal ones and the base of antenna, a pair of small ones on the beak midway between the base of antenna and tip of beak, a pair on the sides of the beak between the latter pair and the tip of the beak, and two pairs on the tip of the beak.

The prothorax is provided with one pair of antero-marginal, setigerous tubercles, one pair of antero-lateral, two pairs of medio-lateral, and four pairs of dorsal setigerous tubercles. The mesonotum and metanotum are each provided with two pairs of spines. The abdomen has seven distinct dorsal tergites, the seventh being somewhat larger than the rest. The dorsal area of each is armed with a pair of large spines and a pair of smaller ones. The lateral area of each tergite is armed with a spine at the base of which is a small seta. The epipleural lobes are each armed with two minute setæ. One pair of the dorsal spines of the seventh abdominal segment is much larger than the rest and is usually directed cephalad; the second pair is small and slender and is directed caudad. The ninth abdominal segment is armed with two fleshy processes.

PLATE 61

Sitophilus linearis:

A.—Pupa, dorsal view.
B.—Pupa, front view.
C.—Egg.
D.—Mandible.
E.—Mature larva.
F.—Ventral view of head.
G.—Clypeus and labrum.
H.—Pupa, lateral view.
I.—Head, face view.
J.—Head, dorsal view.
K.—Head, lateral view


A
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Sc.
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ep.
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al.
Cox
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K
Rc

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE GROWTH OF PSEUDOMONAS CITRI AND ITS HOST PLANTS AND ON INFECTION AND DEVELOPMENT OF THE DISEASE[1]

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INTRODUCTION

In the writer's investigations on the susceptibility and resistance of a large number of rutaceous plants to citrus-canker (*Pseudomonas citri* Hasse) he has been impressed (*7–9*)[2] by a number of factors which appear to play an important rôle in these studies. The factors may be briefly stated as follows:

1. The anatomical structure of the plants.
2. The reaction of the host plants to their environment.
3. The influence of external conditions on the organism and on the susceptibility to infection of the host.
4. The influence of the host on the virulency of the organism.[3]

THE PROBLEM

The problem was attacked from the standpoint of the influence of temperature on the growth of the organism and its hosts and on infection and development of the disease and from the standpoint of the influence of humidity on the growth of the organism and its hosts and on infection and development of the disease.

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[1] Published with the approval of the Director of the Alabama Agricultural Experiment Station as a report on cooperative investigations between the Department of Plant Pathology, Alabama Agricultural Experiment Station, and the Bureau of Plant Industry, United States Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 505–506.

[3] To determine more definitely just what part some of these factors play in governing the susceptibility and resistance of rutaceous plants to canker, leave of four months was granted the writer by the Director of the Alabama Agricultural Experiment Station to carry on this investigation in the Plant Physiology Laboratory at the University of Illinois during the winter of 1918–19. Through the cooperation of Dr. K. F. Kellerman, Associate Chief, Bureau of Plant Industry, United States Department of Agriculture, a second four months' investigation was made possible the following winter. It is indeed with great pleasure that the writer acknowledges his indebtedness to the University of Illinois for the privileges and facilities of the Plant Physiology Laboratory. The writer is especially indebted to Prof. C. F. Hottes for the suggestions, methods, and advice offered during the course of the work and for the time spent by him in preparing, setting up, and regulating the apparatus used. He also wishes to thank Prof. F. L. Stevens for the use of the Plant Pathology Laboratory. The plants used in the experiments were kindly furnished by Mr. W. T. Swingle, in Charge, Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture.

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APPARATUS USED

A complete description of the temperature and humidity cases used in this investigation will soon be published by Prof. Hottes. It is sufficient to state here that the cases were large, well ventilated, well lighted, and most important of all, supplied with accurate and reliable controls. The temperature cases remained constant to within 0.5° C. and were controlled at 5° intervals from 5° to 30°. For work above 30° ordinary bacteriological incubators and one large case held at 35°, but varying several degrees, together with constant-temperature water baths, were used. The cases used for the humidity work were accurate to within 2 to 4 per cent and could be regulated for any desired percentage of relative humidity. The temperature of these cases could also be readily regulated and controlled. Thus, the writer has had the extreme good fortune of working with well-regulated temperature and humidity controls, which were not a continual worry or source of error.

INFLUENCE OF TEMPERATURE ON GROWTH OF THE ORGANISM

Little work has been done on the temperature relations of *Pseudomonas citri*. Doidge (*1*) states that—

> it grows well at 30°C., rather more slowly at 25° C., and very slow progress is made at 20° C.

Wolf (*17*) in preliminary tests found that—

> the thermal death point was between 58° C. and 70° C.

and further that—

> no growth occurred in tubes exposed for temperatures above 65° C.

Stevens (*12*) reports that—

> bacteria (*P. citri*) have been killed by temperatures ranging from 55° C.–60° C., when exposed for a period of five minutes.

Three types of culture media were tested—a liquid, a liquefiable solid, and a solid. These furnished a means of comparing the growth of the organism on different types of media, and if any differences existed between the rate and amount of growth on the different media at various temperatures they could be easily detected. Beef bouillon was used as the liquid, soluble starch agar as the liquefiable solid, and steamed potato cylinders as the solid. Since the most comparable results were obtained with soluble starch agar, they will be taken up first.

SOLUBLE STARCH AGAR.—Hasse (*2*), Wolf (*17*), and Jehle (*5*) have noted the characteristic growth of *Pseudomonas citri* on potato plugs, and especially the formation of a narrow white zone along the margin of the bacterial growth. Doidge (*1*), however, says:

> I have failed to perceive, except in one or two doubtful instances, the narrow white zone on the uninfected surface following the line of the streak in young cultures, which have been recorded both by Hasse and Wolf.

The writer has always noticed this zone on potato plugs, especially in young cultures.

Preliminary tests on inoculated potato plugs with iodin solution showed that the narrow white zone was completely free from starch, while it was surrounded by a small light band of red and blue, indicating that the decomposition of the starch was slowly taking place. In old cultures the cell walls were separated, showing that the middle lamella had been attacked and dissolved. Wolf (*17*) and Doidge (*1*) have reported similar observations. Thus, by the use of soluble starch agar and potato cylinders, the growth of the organism as well as the rate of enzym action at different temperatures could be measured directly.

The soluble starch agar was made up as follows:[1]

12.0 gm. shredded agar.
5.0 gm. soluble starch (Merck), according to Lintner.
.5 gm. potassium phosphate (dibasic).
.5 gm. magnesium sulphate.
.5 gm. sodium chlorid.
1.0 gm. ammonium sulphate.
1.0 gm. calcium carbonate.
1,000 cc. distilled water.

Two methods of measuring the growth of the organism presented themselves: first, the pouring of dilution plates and measuring the growth formed from a single bacterium by means of an enlarged projection through a fixed camera, and second, the placing of a definite amount of inoculum on the agar and measuring the increased diameter of the colony.

The most serious objection to the first method was that the plates could not be poured at the temperatures to which they were subsequently exposed. The minimum temperature for the growth of the citrus-canker organism is approximately 2° to 4° C. lower when this method is used. Also the initial growth at temperatures between 5° and 15° is greater. This is due to the fact that all materials are at room temperature when the inoculations of the plates are made and, furthermore, there is a definite time limit required to bring the plates or tubes to the temperature of that of the case.

In the second method a ½-mm. loop was pressed gently on the hardened agar at three or four points on the plate, so that the inoculum remained on the spot made. The increase in the diameter of the colonies was then measured from day to day. This method is not so accurate from the standpoint of measurement as the first, but it gives much more comparable results, when the temperature and time factors are considered.

All the plates were poured at the same time, care being taken to get the agar in the plates of the same thickness. They were then placed in the various temperature cases overnight, so that at the time of inocula-

[1] A modification of the starch agar used by McBeth and Scales. (McBeth, I. G., and Scales, F. M. The destruction of cellulose by bacteria and filamentous fungi, U. S. Dept. Agr. Bur. Plant Indus. Bul. 266, p. 26–28, 1913.)

tion they were at the temperature of the cases. The inocolum used in all instances was from a 48-hour-old culture of *Pseudomonas citri* in beef bouillon. While the plates were being inoculated precautions were taken to maintain them at the same temperature as that of the case. At the end of every 24 hours two plates were taken from each case, and the increased diameter of the colonies was measured.

In studying the rate of enzym action, an iodin solution[1] was poured over the plate to be tested, was allowed to remain a few moments, and was then poured out. The result was that the colonies stood out as a lemon-yellow color, surrounded by a clear zone which came next showed the disappearance of the starch and its conversion into maltose and achroo-dextrin. This was followed by a reddish band, indicating erythro-dextrin, an intermediate product, which merged into a light blue band and finally into the dark blue color of the remaining agar. Thus, on one plate, both the growth of the colonies and the rate of the enzym action, as indicated by the iodin test, could be measured.

Table I gives the diameter of the colonies in millimeters for each day and temperature. Each reading represents an average of 28 measurements.

TABLE I.—*Diameter in millimeters of colonies of Pseudomonas citri on soluble starch agar at various temperatures*

Temperature.	After 1 day.	After 2 days.	After 3 days.	After 4 days.	After 5 days.	After 6 days.	After 7 days.	After 8 days.
°C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
5	0	0	0	0	0	0	0	0
10	0	0. 25	0. 75	0. 94	1. 24	1. 32	1. 50	1. 63
15	0	0. 51	1. 00	1. 44	1. 94	2. 38	2. 75	3. 38
20	0. 50	1. 50	2. 00	2. 86	3. 25	3. 76	4. 13	4. 50
25	1. 25	2. 37	2. 81	3. 30	4. 06	4. 84	5. 30	5. 81
30	1. 38	2. 63	3. 00	3. 50	4. 50	5. 30	6. 00	6. 38
33 to 35	0	0	0	0	0	0	0	0
38 to 40	0	0	0	0	0	0	0	0

When the time factor, or length of exposure, is considered, the optimum temperature for the growth of *Pseudomonas citri* on soluble starch agar is between 20° and 30° C. There is evidence of a decided lag in the growth of the organism between 15° and 20°. In other words, while the amount of growth at 20° is just one day behind that produced at 25° and two days behind that at 30°, the growth at 15° is much slower, being two days behind the growth made by the organism at 20°. At 20°, growth starts the first day, while at 15°, growth is just starting at the end of the second day. This point is very well brought out in figure 1, where the rate of enzym action at the various temperatures is plotted.

[1] The solution was composed of 0.5 gm. potassium iodid and 1.0 gm. iodin, allowed to stand overnight together in 10 cc. of water. It was then diluted to 100 cc. (stock solution). As needed, the stock solution was diluted to about one-half or less, depending on the material tested.

Growth is inhibited at 5° C. and again at 33° to 35°. At 10° some growth occurs. That the organism is not killed at 5°, but is merely inhibited, was shown when plates kept at this temperature for eight days were transferred to the 30° case. Growth immediately took place at the normal rate for that temperature. The same was true when plates held at 33° to 35° for eight days were transferred to 30°; the organism started growing. However, when plates held at 38° to 40° for 24 hours

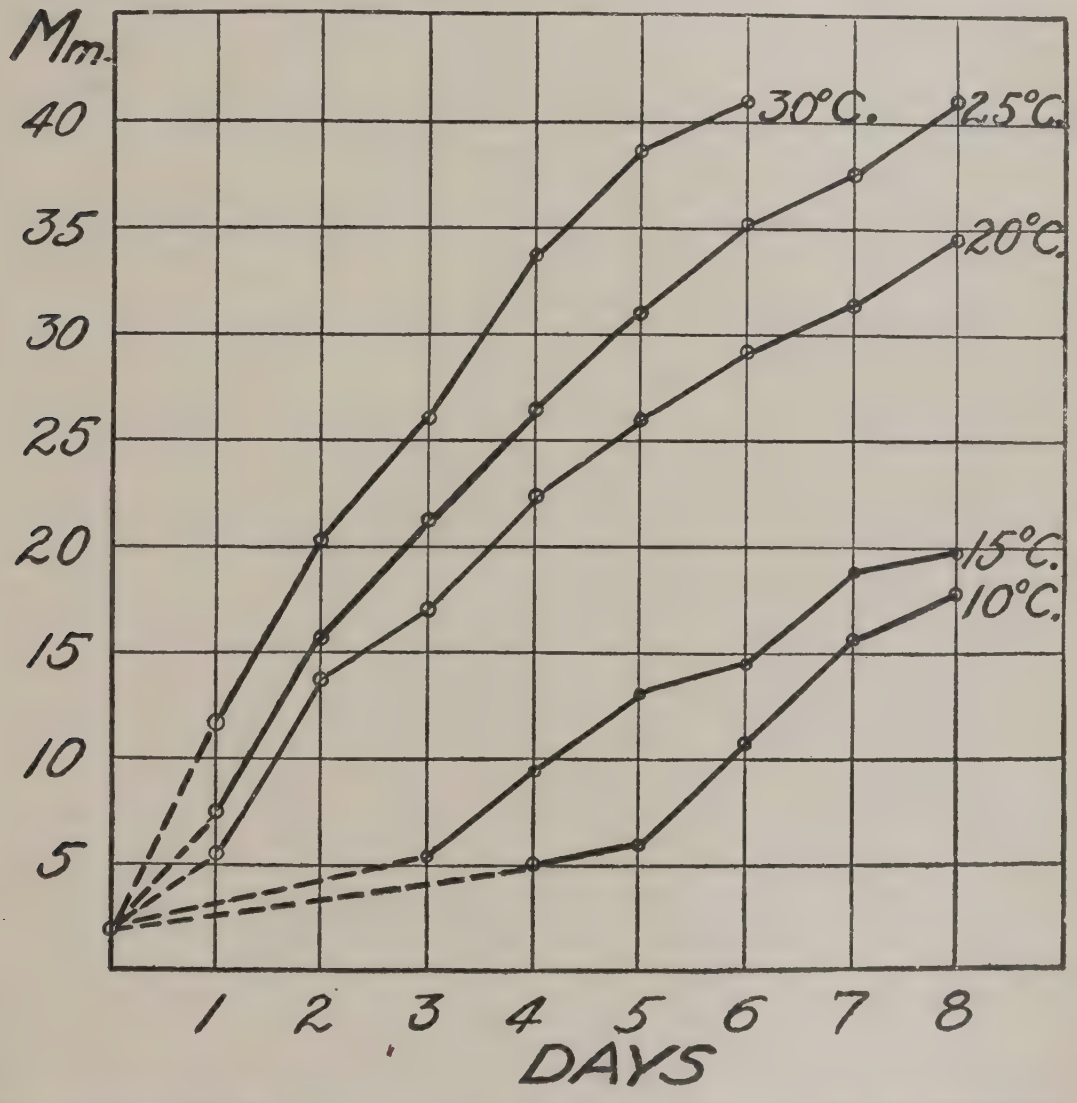


FIG. 1.—Graph showing the rate of enzym action, as expressed in millimeters, at the various temperatures for a period of eight days on soluble starch agar.

were placed in the 30° case, no growth took place, showing that the organism had been killed by the higher temperatures. Thus, in working out the temperature relations of *Pseudomonas citri*, the temperature at which growth is inhibited must be clearly distinguished from the temperature at which the organism is killed.

Table II gives the rate of enzym action at the various temperatures. Each reading represents an average of 28 measurements.

TABLE II.—*Rate of enzym action of Pseudomonas citri on soluble starch agar at various temperatures*

Temperature.	After 1 day.		After 2 days.		After 3 days.		After 4 days.		After 5 days.		After 6 days.		After 7 days.		After 8 days.	
	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.
°C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm*	*Mm.*
5	.....	[a] 5.00	.....	5.00	.....	5.00	.....	5.00	.....	5.00	.....	5.00	.....	5.00	.....	5.00
10	.....	5.00	.....	6.00	.....	6.30	5.00	5.12	6.00	5.38	10.75	4.00	15.62	2.87	17.75	4.75
15	.....	5.12	.....	6.37	5.25	4.75	9.38	3.00	13.00	3.75	14.50	4.20	18.75	3.37	19.75	2.37
20	5.50	1.75	13.75	2.37	17.12	2.53	22.50	3.37	26.00	4.50	29.38	3.62	31.50	4.00	34.50	4.50
25	7.50	1.13	15.63	2.87	21.38	3.37	26.38	3.37	31.12	4.38	35.12	3.25	38.50	4.00	41.00	4.00
30	11.80	1.64	20.12	3.25	26.38	4.49	33.75	3.00	38.50	4.00	41.00	4.00	[b]	.....	.....	.....
33 to 35	.....	[a] 5.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00
38 to 40	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00

[a] Light blue, showing slight hydrolysis of starch.

[b] Discontinued.

At 5° C., 33° to 35°, and 38° to 40° a light blue color was given with the iodin, indicating that only a partial hydrolysis of the starch took place. At 10° the light blue zone persisted for several days, followed by a wide reddish zone. It was not until the fourth day that a clear zone was formed. Likewise, at 15° no clear zone was formed until the third day. At 20°, 25°, and 30° the clear zones were present at the end of 24 hours, increasing in diameter in proportion to an increase in temperature. The curves for the rate of enzym action are shown in figure 1. Especially noticeable are the differences in the rate of enzym action represented by the 15° and 20° curves. The lag mentioned under the rate of growth of the organism at these temperatures is very well shown. Further investigations must be carried out before the explanation of this lag can be given.

POTATO PLUGS.—The first trial with the growth of the organism on potatoes was attempted with blocks of raw potatoes cut under aseptic conditions and placed in Petri dishes with plain agar poured into the dishes even with the top of the blocks to keep them moist. However, this method had to be abandoned because the surface of the blocks oxidized and dried out too rapidly. Therefore in the following trials, steamed potato cylinders were used. The same procedure was followed as in the tests with soluble starch agar to bring the cylinders to the temperature of the cases prior to and during inoculation. They were inoculated by means of a shallow stab, and the organism was allowed to grow out over the surface. The inoculum was taken from a 5-day-old culture of *Pseudomonas citri* on potato plugs. The results are not as comparable as those obtained for starch agar because of the variation in the amount of inoculum and the physical differences in the potato cylinders themselves. However, in general the growth of the organism and the rate of enzym action, as determined by the iodin test, followed the curves shown in figure 1. As the red and blue zone was very narrow on the potato cylinders, the total diameter of the zone is represented in Table III, together with the growth of the organism. This table gives the average of two trials of four readings each.

TABLE III.—*Growth and rate of enzym action of Pseudomonas citri on steamed potato cylinders at various temperatures*

Temperature.	After 1 day.		After 2 days.		After 3 days.		After 4 days.		After 5 days.		After 6 days.		After 7 days.		After 8 days.	
	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.
°C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
5	0	[a] T	0	T	0	T	0	T	0	1.00	T	1.00	T	1.00	T	1.00
10	0	T	T	1.00	1.00	2.00	1.50	2.25	2.00	2.50	2.50	3.00	3.00	4.00	3.50	5.50
15	T	0.50	1.50	2.25	2.00	4.00	2.50	3.50	4.00	5.00	4.00	6.00	4.00	6.00	4.00	7.00
20	0.68	.75	2.50	3.50	4.00	6.00	3.25	7.50	5.50	8.00	6.00	8.00	5.00	9.00	8.00	11.00
25	1.00	1.70	3.75	5.12	6.00	8.00	7.50	10.5	11.00	15.00	Down sides.					
30	2.50	3.50	4.00	5.00	6.50	8.50	12.0	14.5	Down sides.							
33 to 35	0	2.00	0	4.00	0	5.00	0	5.00	0	7.00	0	7.00	0	7.00	0	7.00
38 to 40	0	3.00	0	3.50	0	6.00	0	6.00	0	6.00	0	6.00	0	6.00	0	6.00

[a] T=trace.

At 5° C. a very small zone was noticed after several days, which increased very slowly until at the sixth day the colony was just visible to the naked eye. Growth at 10° was first observed on the third day and increased slowly with time. Growth at 20°, 25°, and 30° was, of course, much more pronounced. No visible growth occurred at 33° to 35° and 38° to 40°, although some enzym action took place.

The surfaces of the cylinders were slightly depressed at 20° C., the depression increasing in depth at 25° and 30°. When the cylinders were cut open, it was found that the clear zone proceeded down in the shape of a cone, and its progress was almost as rapid as that of the zone on the surface.

At 25° and 30° C., where the organism grew over the whole surface and down the sides, the decomposition of the upper half of the plug took place. Examination for starch grains under the microscope showed that none were present, while the middle lamella was completely dissolved, the cells standing apart. From the results of the study of the enzym action of *Pseudomonas citri* on soluble starch agar and steamed potato plugs, we can conclude that it is a strong diastase secretor. Cytase is also produced abundantly.

The organism appeared to thrive longer and produce more enzym near the critical temperatures on potato plugs than it did on the starch agar. At 5° C. a small white zone was produced with a trace of growth. No growth was visible at 33° to 35° or at 38° to 40°, although a rather large depressed zone was distinctly noted. Potato plugs with no visible growth in the 5° and 33° to 35° cases at the end of 8 days produced abundant growth when transferred to 30°. However, plugs kept for 24 hours in the 38° to 40° case when transferred to 30° produced no growth, nor did the white zone increase in size.

BEEF BOUILLON.—All the beef bouillon used in the experiments was adjusted to +8 Fuller's scale, since it was found that the organism developed very well at this acidity. During the course of the work with beef bouillon, no counts were made of the bacterial growth in cultures at the different temperatures.

By means of a bulb burette 10 cc. of the bouillon were placed in each tube. The tubes were kept in the various cases overnight and were inoculated the next morning with a 2-mm. loop from a 48-hour-old culture of *Pseudomonas citri*. Each day two tubes were withdrawn and a reading was taken.

Pseudomonas citri makes a very characteristic growth in beef bouillon. Growth is first noticed by the clouding of the medium. After a few days, flakes appear, followed by a yellow ring at the surface of the bouillon; later, the flakes precipitate to the bottom. Thus, in Table IV, the readings are based on the characteristic behavior of the organism.

TABLE IV.—*Growth of Pseudomonas citri in beef bouillon at various temperatures*

Temperature.	After 1 day.	After 2 days.	After 3 days.	After 4 days.	After 5 days.	After 6 days.	After 7 days.	After 8 days.
° C.								
5	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear.
10	do	do	Very slightly cloudy.	Slightly cloudy	Flaking	Flaking	Flaking	Flaking.
15	do	Very slightly cloudy.	Flaking	Flaking	do	Ring	Ring	Ring.
20	Very slightly cloudy.	Slightly cloudy	do	Flaking. Ring	Precipitate	Precipitate	Precipitate	Precipitate.
25	Slightly cloudy	Flaking	Flaking. Ring	Precipitate	do	do	do	Do.
30	do	Flaking. Ring	Precipitate	do	do	do	do	Do.
33 to 35	do	Slighty cloudy	Slightly cloudy	Slightly cloudy	Slightly cloudy	Slightly cloudy	Slightly cloudy	Slightly cloudy.
38 to 40	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear.

No visible growth occurred at 5° C. or at 38° to 40°. Growth was visible at 10° the third day, at 15° the second day, and at 20°, 25°, 30°, and 33° to 35° at the end of 24 hours. However, at 33° to 35° no increase in growth was noted after the first day.

Growth proceeded at a regular rate at 20° C., 25°, and 30°, each of the lower temperatures being about one day behind. Growth at 15° never approached that produced at 20°. The growth of *Pseudomonas citri* in beef bouillon at the different temperatures proceeded at the same relative rate as on agar and potato plugs.

Cultures placed in the 5° C. and the 33° to 35° cases were viable at the end of 8 days, while no organisms were viable in tubes held at 38° to 40° for 24 hours. In constant water baths, the organisms were viable at 37.6° to 38° at the end of 24 hours and at 41° to 41.5° at the end of 2 hours. At 43.2° to 43.6° all organisms were dead at the end of 2 hours. The interesting point brought out here is that no organisms were viable in the incubators run at 38° to 40° C. at the end of 24 hours, but at 37.6° to 38° in the water bath they were viable at the end of 24 hours. Thus, the point at which growth is completely inhibited at the higher temperatures is very sharp when the time factor is constant.

In determining the thermal death point by the usual laboratory methods, all the organisms were killed at a temperature of 52° C., while all the trials at 49° yielded positive results. Thus, the thermal death point of the citrus-canker organism is above 49° and below 52°.

Distilled water.—To determine the length of life of *Pseudomonas citri* in water, ordinary distilled water, supplied by the Department of Chemistry, University of Illinois, was used, as it was impossible at the time to obtain good well or rain water. The distilled water contained traces of organic matter, but no mineral substances were present. No attempts were made to determine the conductivity of this water.

The sterilized water blanks were placed in the cases at the various temperatures overnight and were inoculated with a loop from a 48-hour-old culture of the organism in beef bouillon. At the end of each day, two tubes were withdrawn from each case and were reinoculated into beef bouillon to test for growth. In Table V the results of the experiment are given.

Table V.—*Viability of Pseudomonas citri in sterilized distilled water at various temperatures*

Temperature.	After 1 day.		After 2 days.		After 3 days.		After 4 days.		After 5 days.		After 6 days.		After 7 days.		After 8 days.	
°C																
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The results show very clearly that *Pseudomonas citri* can remain viable in the distilled water used for a period of eight days at temperatures ranging from 10° to 35° C. They suggest that the citrus-canker organism under certain field conditions may remain viable in rain and surface water for some time at a range of temperatures much larger than is usually found in the field.

Comparative tests with the organism in beef bouillon and in distilled water at temperatures higher than 35° C. gave the same results. For example, the thermal death point of the organism in the distilled water was between 49° and 52°, just as in beef bouillon.

CONCLUSIONS ON THE TEMPERATURE RELATIONS OF THE ORGANISM

(1) The optimum temperature for the growth of *Pseudomonas citri* on soluble starch agar, potato cylinders and in beef bouillon lies between 20° and 30° C.

(2) There is a decided lag between the rate of growth at 15° C. and that at 20° in all media.

(3) The minimum temperature for the growth of *Pseudomonas citri* is 5° C. on potato plugs. However, growth on soluble starch agar and in beef bouillon is inhibited at this temperature, so that the minimum temperature for the growth on these media must be slightly above 5°.

(4) The maximum temperature for the growth of *Pseudomonas citri* in beef bouillon is 43° C. for periods of less than 2 hours, 41° for a period of 2 hours, 38° for a period of 24 hours, and 33° to 35° for periods longer than 24 hours. Growth on potato cylinders and soluble starch agar was, in all cases, inhibited at temperatures of 33° to 35°, so that the maximum temperature for the growth on these media must be slightly below 33° to 35°.

(5) The thermal death point of the organism is above 49° and below 52° C.

(6) The temperatures at which growth is inhibited must be clearly distinguished from the temperatures at which the organism is killed. This is especially important near the critical temperatures at or above the maximum. The point at which growth is completely inhibited at the higher temperatures is very sharp with a constant length of exposure.

(7) The production of diastase by *Pseudomonas citri* on soluble starch agar and potato cylinders follows the well-known chemical law of Van't Hoff, between temperatures of 20° and 30° C. As in the growth of the organism, there is a decided lag between the rate of enzym action at 15° and that at 20°. This fact has not been pointed out heretofore. Only partial hydrolysis of the starch in the agar and the potato cylinders occurs at 5° and again at 33° to 35° and 38° to 40°.

(8) The citrus-canker organism is viable in ordinary distilled water at temperatures ranging from 10° to 35° C. for a period of eight days.

INFLUENCE OF TEMPERATURE ON GROWTH OF THE HOST PLANTS

The literature on the influence of the environmental conditions on the growth and development of Citrus plants is very meager. What literature is available concerns itself chiefly with the injury to Citrus orchards caused by low temperatures, with an occasional reference to the maximum temperatures at which the Citrus plants can thrive.

The most complex factor entering into the study of the temperature relations of Citrus plants is the fact that they have rest and growth periods which vary to some extent with each group, although they are more or less definite within the group itself. Under greenhouse conditions, the rest and growth periods are variable. However, as a general rule, most Citrus plants can be forced into active growth within short periods of time. An exception to this statement must be made for deciduous plants like *Poncirus trifoliata*. With plants of this type, external conditions in the greenhouse have no influence on the rest period, within certain limits.

Three types of plants were used—*Poncirus trifoliata* (L.) Raf. and Rusk citrange (a hybrid between *P. trifoliata* and *Citrus sinensis* Osbeck, Florida sweet orange), plants which are deciduous, hardy, susceptible to citrus-canker, and having a very definite dormant period; *C. grandis* (L.) Osbeck, grapefruit, an evergreen and nonhardy plant, extremely susceptible to citrus-canker and having a dormant period of variable nature; and *C. mitis* Blanco, calamondin, an evergreen and nonhardy plant, somewhat resistant to citrus-canker, and native of the Philippine Islands.

The plants were grown from seed in the Crop Physiology greenhouses at Washington, D. C. The seedlings ranged from 6 to 14 inches in height and were shipped from Washington from time to time, both in pots and balled. Several shipments of *Poncirus trifoliata* were made of seedlings growing outside, from Auburn, Ala., during the month of January. The plants were kept under greenhouse conditions until needed.

In the experiments reported below, the plants were placed under large bell jars in the various temperature cases. During the course of the experiments, a saturated atmosphere was maintained in the bell jars. Observations and readings were made of the condition of the plants from time to time.

EXPERIMENT I

Two plants of each species were placed in the cases at the various temperatures, while one set was kept under greenhouse conditions where the temperature range was considerable, varying from 20° to 30° C. All plants, with one or two exceptions, were either in a dormant state or had completed their growth. In Table VI are given the observations made on the plants at intervals for a period of six weeks.

TABLE VI.—*Growth of three representative Citrus plants at various temperatures*

EXPERIMENT 1

Temperature.	Date of reading.	Number of days.	*Poncirus trifoliata.*		*Citrus mitis.*		*Citrus grandis.*	
			Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.
°C.								
10	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Complete	Complete.
	Jan. 31, 1919	42	No change	No change	No change	No change	No change	No change.
15	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Complete	Complete.
	Jan. 13, 1919	23	No change	No change	No change	No change	New growth	New growth.
	Jan. 20, 1919	31	do	do	do	do	do	Complete.
	Jan. 24, 1919	35	do	do	do	do	Complete	Do.
	Jan. 28, 1919	39	do	do	do	do	New growth	New growth.
	Jan. 31, 1919	42	do	do	do	do	do	Do.
20	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Good	Good.
	Jan. 4, 1919	14	No change	No change	No change	No change	Excellent [a]	Excellent.
	Jan. 13, 1919	23	do	do	do	do	Excellent	Complete.[a]
	Jan. 20, 1919	31	do	do	do	do	Complete	New growth.[a]
	Jan. 24, 1919	35	do	do	do	do	New growth [a]	Good.[a]
	Jan. 28, 1919	39	do	do	do	do	Good [a]	Complete.
	Jan. 31, 1919	42	do	do	do	do	Complete [a]	Do.
25	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Good	Good.
	Jan. 4, 1919	14	No change	No change	No change	No change	New growth	Complete.
	Jan. 9, 1919	19	do	do	No change [a]	do	do	Do.
	Jan. 13, 1919	23	do	do	No change	do	New growth [a]	Do.
	Jan. 20, 1919	31	do	do	do	do	do	Do.
	Jan. 24, 1919	35	do	do	do	do	Excellent [a]	Complete.[a]
	Jan. 28, 1919	39	do	do	No change [a]	No change [a]	Excellent	Complete.
	Jan. 31, 1919	42	do	do	do	No change	Excellent [a]	Complete.[a]
30	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Complete	Complete.
	Jan. 4, 1919	14	No change	No change	No change	No change	New growth [a]	New growth.
	Jan. 6, 1919	16	do	do	No change [a]	do	New growth	Do.
	Jan. 9, 1919	19	do	do	do	No change [a]	do	New growth.[a]
	Jan. 13, 1919	23	do	do	New growth	New growth	do	New growth.
	Jan. 20, 1919	31	do	do	Complete [a]	New growth [a]	do	Complete.
	Jan. 24, 1919	35	do	do	do	do	Complete	Do.
	Jan. 28, 1919	39	do	do	Complete	New growth	do	Do.
	Jan. 31, 1919	42	do	do	Complete [a]	New growth [a]	New shoot	Do.

Greenhouse, control.	Dec. 20, 1918	0	Dormant	Dormant	Good	Good	Good.
	Jan. 4, 1919	14	No change	No change	Complete [a]	Complete	Do.
	Jan. 6, 1919	16	do	do	do	New growth	New growth.
	Jan. 9, 1919	19	do	do	New shoot	do	New growth. [a]
	Jan. 13, 1919	23	do	do	do	do	New growth. [a]
	Jan. 20, 1919	31	do	do	do	do	New growth.
	Jan. 24, 1919	35	do	do	Excellent	Excellent	Excellent.
	Jan. 28, 1919	39	do	do	Excellent [a]	New growth	New growth. [a]
	Jan. 31, 1919	42	do	do	Excellent	New growth [a]	Do.

[a] New spots.

It will be noted that, even in a saturated atmosphere, the various temperatures had no influence whatsoever on the dormant plants of the trifoliate orange. No growth of the calamondin plants occurred except at 30° C. and in the greenhouse.

At 10° C. no growth of the grapefruit plants took place. It is very evident that at 15° the growth of grapefruit is not only slow but that the growth matures very rapidly. Leaves which mature at this temperature are small, being from one-fourth to one-half the size of the normal grapefruit leaf. Good growth of the grapefruit plants took place at 20° C. However, the shoots did not grow so rapidly and the maturation of the leaves was faster than at the higher temperatures of 25° and 30°. At these temperatures, where the grapefruit plants were in good condition, a rapid growth took place, the new shoots were longer, and the period over which the maturation of the leaves took place was extensive. To illustrate, at 15° it required from 7 to 8 days for a new shoot to complete its growth, while at 30°, 16 to 20 days were necessary.

EXPERIMENT 2

In this experiment plants of the Rusk citrange were substituted for the trifoliate orange. Three plants of the citrange, three of the calamondin, and one of the grapefruit were used. One plant each of the citranges and calamondins, in a good growing condition, was chosen for the first group; one set in which the growth was complete, but with a new bud starting, was selected for the second group; and dormant plants were placed in the third group. Grapefruit plants in good growing condition were used. The experiment was carried through in the same way as experiment 1, except that at the end of 15 days the plants in the 5°, 10°, and 15° C. cases were all transferred to the 30° case under their original bell jars.

During the 15-day period no growth of the citrange, calamondin, and grapefruit plants occurred at 5° and 10° C. (Table VII.) An extremely slow growth was recorded for the grapefruit plants at 15°. Measurements of two grapefruit leaves showed an increase in growth of 3 mm. and 9 mm. in length and 1 mm. and 4 mm. in width, respectively, for a period of 15 days. As noted in experiment 1, leaf maturity increased very rapidly at these temperatures, the leaves reaching about one-fourth to one-half the size of those at higher temperatures.

When the plants held at temperatures of 5° and 10° C. were placed in the 30° case, a normal growth for that temperature immediately took place in most instances. The rate of growth of the growing citranges was about 25 mm. per day. The behavior of the dormant plants when transferred to the higher temperatures was erratic. Some immediately responded and started growth, while others remained dormant.

TABLE VII.—*Growth of Citrus plants at various temperatures*

EXPERIMENT 2

Temperature.	Date of reading.	Number of days.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*
			Good growing condition.	Complete, new bud starting.	Dormant.	Good growing condition.	Complete, new bud starting.	Dormant.	
°C.	1919.								
5	Jan. 16	0	Starting, 7 mm.	Starting, 2 mm.	Dormant.	2 shoots.	Complete.	Dormant.	2 shoots.
	Jan. 31[a]	15	No change.	No change.	No change.	Mature.	do.	do.	No change.
	Feb. 5	5	Good, 45 mm.	Good, 25 mm.[b]	Dormant.	3 shoots.	do.	Starting.	Good.[b]
	Feb. 8	8	Good, 70 mm.[b]	Good, 55 mm.[b]	do.	Good.	do.	do.	Excellent.[b]
	Feb. 12	12	Good, 100 mm.	Good, 80 mm.	do.	5 shoots.	do.	do.	Do.
10	Jan. 16	0	Starting, 10 mm.	Starting, 3 mm.	do.	2 shoots.	Good.	Dormant.	1 shoot.
	Jan. 31[a]	15	No change.	No change.	No change.	No change.	Mature.	do.	No change.
	Feb. 5	5	Good, 35 mm.[b]	Starting, 9 mm.	Starting, 5 mm.	3 shoots[b]	do.	Starting.	Excellent.[b]
	Feb. 8	8	Good, 55 mm.[b]	Good, 30 mm.[b]	Good, 17 mm.	Excellent[b]	do.	do.	Excellent.
	Feb. 12	12	Good, 70 mm.[b]	Good, 60 mm.[b]	Good, 40 mm.[b]	Excellent.	Complete.	do.	Excellent.[b]
15	Jan. 16	0	Starting, 7 mm.	Starting, 2 mm.	Dormant.	1 shoot.	do.	Dormant.	2 shoots.
	Jan. 24	8	No change, 10 mm.	No change, 2 mm.	do.	No change.	do.	do.	Do.
	Jan. 28	12	do.	do.	do.	do.	do.	do.	Do.
	Jan. 31[a]	15	do.	do.	do.	do.	do.	do.	Do.
	Feb. 5	5	Good, 30 mm.[b]	Starting, 4 mm.	do.	3 shoots.	do.	Starting.	Excellent.[b]
	Feb. 8	8	Good, 45 mm.[b]	Good, 10 mm.[b]	do.	Good[b]	do.	do.	Do.
	Feb. 12	12	Good, 70 mm.[b]	Good, 40 mm.	do.	do.	do.	Good.	Do.
20	Jan. 16	0	Starting, 10 mm.	Starting, 2 mm.	do.	1 shoot.	do.	Dormant.	1 shoot.
	Jan. 20	4	Good, 25 mm.	Good, 8 mm.	Starting, 6 mm.	Good.	do.	do.	Good.[b]
	Jan. 24	8	Good, 40 mm.	Good, 10 mm.	Starting, 10 mm.	do.	do.	do.	Good.
	Jan. 28	12	Good, 60 mm.	Good, 20 mm.	Good, 20 mm.	Complete.	do.	do.	Complete.[b]
	Jan. 31	15	Good, 60 mm.[b]	Good, 25 mm.[b]	Good, 25 mm.	do.	do.	do.	Complete.
	Feb. 5	20	Complete, 60 mm.	Good, 30 mm.	Good, 30 mm.	do.	do.	do.	Do.
	Feb. 8	23	do.	Complete, 30 mm.	Complete, 30 mm.	do.	do.	do.	Do.
	Feb. 12	27	do.	do.	Complete, 30 mm.[b]	do.	do.	do.	Do.
25	Jan. 16	0	Starting, 6 mm.	Starting, 2 mm.	Dormant.	1 shoot.	do.	Starting.	2 shoots.
	Jan. 20	4	Starting, 20 mm.	Starting, 10 mm.	Starting, 8 mm.	Good.	Starting.	Starting.	2 shoots.[b]
	Jan. 24	8	Starting, 40 mm.[b]	Starting, 15 mm.[b]	Starting, 10 mm.[b]	Good[b]	Good.	Good.	2 shoots.
	Jan. 28	12	Good, 40 mm.[b]	Good, 30 mm.[b]	Good, 30 mm.[b]	Good.	Excellent.	do.	Do.
	Jan. 31	15	Complete, 40 mm.	Complete, 30 mm.	Complete, 30 mm.	Good[b]	do.	do.	Do.
	Feb. 5	20	Complete, 40 mm.[b]	Complete, 30 mm.[b]	Complete, 30 mm.[b]	Excellent.	Complete.	Complete.	Do.
	Feb. 8	23	Complete, 40 mm.	Complete, 30 mm.	Complete, 30 mm.	do.	do.	Starting.	Poor.
	Feb. 12	27	do.	do.	do.	Excellent[b]	do.	Good.	Do.

[a] Transferred to the 30° C. case.

[b] New spots.

TABLE VII.—*Growth of Citrus plants at various temperatures*—Continued

EXPERIMENT 2—continued

Temperature.	Date of reading.	Number of days.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*
			Good growing condition.	Complete, new bud starting.	Dormant.	Good growing condition.	Complete, new bud starting.	Dormant.	
°C.	1919.								
30	Jan. 16	0	Starting, 4 mm.	Starting, 2 mm.	Dormant.	1 shoot.	Complete.	Dormant.	1 shoot.
	Jan. 20	4	Good, 35 mm.	Good, 20 mm.	Starting, 18 mm.	Excellent [b]	do.	Starting [b]	4 shoots.[b]
	Jan. 24	8	Good, 60 mm.[b]	Good, 55 mm.[b]	Good, 50 mm.[b]	Excellent.	do.	Starting.	Excellent.[b]
	Jan. 28	12	Good, 80 mm.[b]	Good, 80 mm.[b]	Good, 65 mm.[b]	Excellent [b]	Complete [b]	Good.	Excellent.
	Jan. 31	15	Good, 85 mm.[b]	do.	Good, 70 mm.[b]	do.	do.	Good [b]	Excellent.[b]
	Feb. 5	20	Complete, 85 mm.	Good, 85 mm.[b]	Good, 75 mm.	Excellent.	do.	do.	Do.
	Feb. 8	23	Complete.	Complete, 85 mm.	Complete, 75 mm.	Excellent [b]	do.	Good.	Do.
	Feb. 12	27	Complete [b]	Complete [b]	Complete [b]	do.	do.	do.	Do.
Greenhouse, control	Jan. 16	0	Starting, 6 mm.	Starting, 2 mm.	Dormant.	1 shoot.	Complete.	Dormant.	Complete.
	Jan. 20	4	Starting, 20 mm.	Starting, 10 mm.	Starting, 3 mm.	do.	do.	Starting.	Do.
	Jan. 24	8	Good, 40 mm.	Good, 35 mm.	Good, 20 mm.	Complete.	do.	do.	3 shoots.
	Jan. 28	12	Good, 60 mm.	Good, 50 mm.	Good, 25 mm.	do.	do.	Good [b]	4 shoots.
	Jan. 31	15	Good, 70 mm.	Good, 65 mm.	Good, 35 mm.[b]	New growth.	do.	Excellent [b]	Excellent.
	Feb. 5	20	Excellent, 90 mm.[b]	Good, 70 mm.[b]	Good, 35 mm.	do.	Starting.	8 shoots.	5 shoots.
	Feb. 8	23	do.	do.	Complete.	Good.	Good.	Excellent.	Excellent.[b]
	Feb. 12	27	Excellent.	Good.	do.	do.	do.	do.	Do.

[b] New spots.

In no instance were the small, undersized leaves, which were pushed to maturity at the low temperatures, affected when transferred to a higher temperature. Thus, a leaf that has once reached its maturity can not be made to increase in size by a change of environment.

At the temperatures of 20°, 25°, and 30° C., growth responded at a normal rate where the citrange and grapefruit plants were in active condition. In general, no differences were noted in the rate of growth at these temperatures. Thus, the optimum temperature is between these points for the plants named above. With one exception, all dormant grapefruit and citrange plants were forced into active growth. The plants and leaves also made a rapid and large growth and reached maturity rather slowly. Not much difference was noted between the plants kept as controls under greenhouse conditions and those grown at the temperatures named. Apparently, calamondin has a little higher optimum temperature, since little or no growth occurred at 20°.

EXPERIMENT 3

This experiment was carried out with a view of determining the rate of growth under a varying day and night temperature. Thus, plants were exposed during the day at 30° C., and during the night three different sets of plants were placed at temperatures of 10°, 15°, and 20°. The bell jars with the plants were shifted from the 30° case at 5 p. m. and replaced at 8 a. m. the next day.

Two plants each of the trifoliate orange, calamondin, grapefruit, and one of the Rusk citrange were used in each set. The experiment was carried out under the same conditions as the others described above.

As will be noted in Table VIII, the plants held at 30° C. throughout the experiment produced the most growth. Where a day temperature of 30° and a night temperature of 20° were used, there was a very slight slowing down of the growth in all plants except the grapefruit. When night temperatures of 15° and 10° were used, there was a decidedly slower growth. However, growth was not checked, especially with the rapidly growing grapefruit plants. The maturation of the leaves was also more rapid at the low night temperatures. Thus, a night temperature lower than that at which growth normally occurs merely slows up the growth somewhat so long as a high day temperature prevails; it does not completely stop the growth of the trifoliate orange, citrange, and calamondin plants. Little or no difference could be detected in the rate of growth of the grapefruit plants at the different night temperatures. Leaf maturity was hastened somewhat by low night temperatures.

EXPERIMENT 4

It was found in experiment 3 that an alternating day and night temperature inhibited the growth of the trifoliate orange, Rusk citrange, and the calamondin plants, while little or no difference could be detected in the rate of growth of the grapefruit at the different night temperatures. To determine the effect on growth of an alternating temperature for longer periods, plants were started at a high temperature, then placed at a low temperature for about three weeks, and then transferred back to the higher temperature

Two sets of plants in approximately the same condition consisting of one Rusk citrange, one calamondin, and two grapefruit plants (one large plant and one just starting new growth) were used. The first set was retained at 30° C. as a control. The second set after being held at 30° for 24 hours was placed in the 15° case for 18 days and then was finally transferred back to the 30° case for approximately 2 weeks. The results of the experiment are given in Table IX.

TABLE VIII.—*Growth of Citrus plants held at a high temperature during the day and a lower temperature at night*

EXPERIMENT 3

Temperature.	Date of reading.	Number of days.	*Poncirus trifoliata.*		Rusk citrange.	*Citrus mitis.*		*Citrus grandis.*	
			Plant No. 1.	Plant No. 2.		Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.
	1919.								
30° C., control	Feb. 13	0	2 shoots	Good	2 shoots	Good	Good	Good	2 shoots.
	Feb. 20	7	Excellent	Excellent[a]	Excellent[a]	Excellent[a]	Excellent[a]	Complete[a]	Excellent.[a]
	Mar. 2	18	Excellent[a]	do	do	do	do	do	Do.
Day, 30° C.; night, 20°	Feb. 13	0	Good	Good	Good	Good	2 shoots	Good	Good.
	Feb. 20	7	Mature	Mature	Mature	Mature	Excellent	Mature	Mature.
	Mar. 2	18	Complete[a]	Complete	Complete	Complete	Complete	2 shoots[a]	1 shoot.[a]
Day, 30° C.; night, 15°	Feb. 13	0	Good	Good	Good	Good	do	Good	Good.
	Feb. 20	7	Mature	Mature	Mature	Mature	do	Mature	Mature.
	Mar. 2	18	Complete	Complete[a]	Complete	Complete	2 shoots[a]	Complete[a]	Complete.[a]
Day, 30° C.; night, 10°	Feb. 13	0	Good	Good	Good	Good	Complete	1 shoot	1 shoot.
	Feb. 20	7	Mature	Mature	Mature	Mature	do	do	Do.
	Mar. 2	18	Complete	Complete	Complete	Complete	do	Excellent[a]	Excellent.[a]

[a] New spots.

TABLE IX.—*Influence of alternating high and low temperatures on the growth of Citrus plants*

EXPERIMENT 4

Temperature.	Date of reading.	Number of days.	Rusk citrange.	*Citrus mitis.*	*Citrus grandis.*	
					Plant No. 1.	Plant No. 2.
° *C.*	1919.					
30, control.	Dec. 10	0	Starting	New growth	New growth	New growth starting.
	Dec. 12	2	Shoot, 30 mm	do	New growth [a]	Do.
	Dec. 14	4	Shoot, 60 mm [a]	New growth rapid [a]	3 twigs [a]	New growth (4 leaves).[a]
	Dec. 20	10	Shoot, 90 mm.[a]	do	Growth rapid [a]	New growth rapid.[a]
	Dec. 29[b]	19	Shoot, 110 mm.[a]	Some leaves maturing [a]	Some leaves maturing [a]	Leaves maturing.[a]
30	Dec. 10[c]	0	Starting	New growth	New growth	Starting.
15	Dec. 11	0	No change	No change	No change	No change.
	Dec. 14	3	do	do	do	Do.
	Dec. 20	9	do	do	No change [a]	Do.
	Dec. 29[d]	18	do	do	No change	Do.
30	1920.					
	Jan. 6	9	do	Growth rapid	Growth rapid [a]	Growth rapid.[a]
	Jan. 10	13	do	Growth rapid [a]	do	Do.

[a] New spots. [b] Experiment discontinued. [c] Transferred to 15° C. case. [d] Transferred to 30° C. case.

The plants which served as controls all made a rapid growth. At 15° C. the plants were all inhibited in their growth, there being practically no change during the interval the plants were held at this temperature. The leaves of the large grapefruit plant grew slowly and began to mature.

Immediately on being transferred back to the 30° C. case, all but one plant proceeded to grow rapidly at the normal rate for this temperature. Thus, a temperature of 15° has a very decided inhibiting effect on the growth of Citrus plants, much more so than in experiment 3, where the plants were subjected to a temperature of 30° during the day and 15° and lower at night.

EXPERIMENT 5

In the preceding experiments, it has been clearly demonstrated that the most growth of all Citrus plants tested occurs at 30° C. Likewise, the best development of the organism in culture occurred at this same temperature. Above this temperature, growth of the organism was more or less inhibited. Thus, to determine what effect temperature higher than 30° would have on the growth of the plants, the following experiment was carried out. Plants in various stages of growth, as shown in Table X, were divided into four sets and placed in a saturated atmosphere under bell jars at a temperature of approximately 35°. The results show very decidedly that grapefruit and the other plants of this same type were distinctly inhibited by this temperature, even though actively growing plants were used. However, after they were transferred to the 30° case, the young growth started out at the normal rate for that temperature.

On the other hand, the trifoliate orange and limequat [1] plants made a good growth at 35° C. It is interesting to note that this is just the opposite of the result obtained at lower temperatures. Grapefruit was able to make a slow growth at 15°, while the trifoliate orange and calamondin plants were unable to develop at all.

[1] A hybrid between *Citrus aurantifolia*, West Indian lime, × *Fortunella japonica*, round kumquat.

TABLE X.—*Growth of Citrus plants at 35° C.*

EXPERIMENT 5

Set No.	Date of reading.	Number of days.	*Poncirus trifoliata.* Plant No. 1.	*Poncirus trifoliata.* Plant No. 2.	Limequat.	Sweet lemon.	Ruby orange.	Tangelo.	Sour orange.	Grapefruit.
	1920.									
	Feb. 22	0	Dormant	Starting	Starting	Complete	Complete	Complete	Complete	New growth.
	Feb. 27	5	New shoot, 10 mm.	2 shoots, 20 mm.[a] and 22 mm.	3 shoots	do	do	do	do	Maturing.
1	Mar. 4	11	New shoot, 30 mm.	2 shoots, 40 mm.[a] and 75 mm.	3 shoots [a]	do	do	do	do	Maturing.[a]
	Mar. 15	22	Excellent	Excellent	do	do	do	do	do	Complete.
	Feb. 22	0	Dormant	New shoot	Starting	do	do	do	do	Starting.
	Feb. 27	5	do	New growth, 20 mm.	2 shoots	do	do	do	Starting	No change.
2	Mar. 4	11	do	2 shoots, 40 mm.[a] and 55 mm.	Good [a]	Complete [a]	do	do	No change	Do.
	Mar. 15	22	do	Excellent [a]	Good	Complete	do	do	do	Do.
	Feb. 22	0	do	Starting	Starting	do	do	do	Complete	Starting.
	Feb. 27	5	2 shoots, 25 mm. and 20 mm.	1 shoot, 30 mm.[a]	5 shoots	Starting	do	Starting	Starting	No change.
3	Mar. 4	11	2 shoots, 55 mm. and 35 mm.	2 shoots, 35 mm. and 60 mm.[a]	Good	No change	do	No change	No change	Do.
	Mar. 15	22	Excellent	Excellent	Good [a]	do	do	do	do	Do.
	Feb. 22	0	Dormant	Starting	Dormant	Complete	do	Starting	Starting	New growth.
	Feb. 27	5	Starting	2 shoots, 22 mm. and 22 mm.	Starting	do	do	No change	New growth, 20 mm.	No change.
4	Mar. 4	11	New shoot, 45 mm.	2 shoots, 55 mm. and 50 mm.	No change	do	do	do	New growth, 45 mm.	Do.
	Mar. 15	22	Excellent	Excellent	do	do	do	do	No change	Do.

[a] New spots.

It can be concluded from this experiment that the growth of grapefruit and plants of a similar type is decidedly inhibited at a temperature of 35° C., while the trifoliate orange and limequat can make a normal growth, at least for the period of time covered by the experiment.

CONCLUSIONS ON THE TEMPERATURE RELATION OF THE HOST PLANTS

(1) With actively growing *Citrus grandis* plants in a saturated atmosphere the optimum temperature lies between 20° and 30° C. The lower limit of the optimum temperature is a little higher for *C. mitis*, while for *Poncirus trifoliata* and allied plants the upper range of the optimum temperature is above 30°.

(2) No temperature used was able to force the dormant *Poncirus trifoliata* plants into active growth.

(3) The minimum temperature for the growth of *Citrus grandis* is 15° C., and for the others tested it was 20°.

(4) *Citrus grandis* plants kept at a temperature of 15° C. matured their foliage very rapidly and in most instances within a week's time. At temperatures of 20° and above, growth was more rapid and extensive. The period of maturation of the leaves was extended so that 16 to 20 days or more were required, which is twice as long as at 15°.

(5) Leaves that have once reached their maturity at low temperatures can not be forced to increase their size by a change of environment.

(6) A low night temperature checks the growth of plants held at a high temperature during the day and also hastens maturation of the leaves. This is especially noticeable with the *Poncirus trifoliata*, citrange, and *Citrus mitis* plants. *C. grandis*, on the other hand, is not so easily influenced.

(7) Plants grown at a high temperature are inhibited in their growth when transferred to low temperatures. *Citrus grandis* is only slightly inhibited, while *Poncirus trifoliata*, Rusk citrange, and *Citrus mitis* plants are completely checked.

(8) Growth of *Citrus grandis* and plants of a similar type is decidedly inhibited at a temperature of 35° C., while *Poncirus trifoliata* and limequat make a normal growth, at least for the period of experiment.

INFLUENCE OF TEMPERATURE ON INFECTION AND DEVELOPMENT OF THE DISEASE

In discussing infection and development of citrus-canker, two factors have been stressed by the workers in this field. Both have been given equal prominence and can not very well be dissociated. These factors are weather conditions and the condition of the host plant. In discussions of weather conditions, most of the emphasis has been placed on humidity as favoring the more rapid development of the disease, while little has been said regarding the influence of temperature. However, it has usually been inferred that a favorable temperature for infection existed.

Since the literature on the influence of temperature can not be discussed separately from that of humidity, a brief review of the literature on the relation of weather conditions and the condition of the host plant on infection and development of citrus-canker will be given at this point.

Both Hasse (*2*) and Doidge (*1*) found that the disease developed most rapidly on inoculated plants in a saturated atmosphere kept at 30° C.

Stevens (*11, 12*) makes the following statements:

In this experiment, it was found that considerable moisture must be present before infection took place, and in many cases, the small trees thus treated had to be kept drenched and under bell-jars for two or three days. Infections developed slowly under greenhouse conditions, and were fewer in number than those obtained in the open.

Warm humid weather favors rapid development of the disease and thus it is more destructive during the rainy season.

The disease develops and spreads rapidly during rainy weather but it is more or less retarded during periods of drought or in dry weather.

High temperatures and high humidity favor a rapid development and spread of Citrus-canker and these are the prevailing factors of the Florida climate.

Stirling (*15*) states that—

during warm, wet periods, the disease infects quickly and matures in a few days.

Further that—

during a time when the atmosphere is humid, in the rainy season, it spreads rapidly. I have found that during the early part of the season, it requires two or three months for the canker to infect and mature so as to reproduce itself, owing, no doubt, to the dryness and coolness of the weather. Under favorable conditions, however, the canker will infect and mature in a much less time.

Wolf (*17*) observed that—

the most rapid development of the disease occurred under humid conditions.

Jehle (*4, 5*) in a number of articles states:

Citrus-canker is one of the most destructive diseases of citrus plants . . . and especially where the climate is warm and moist during part or all of the year,—as the disease develops most rapidly when the humidity is high . . . it was most severe and the incubation period shortest during warm moist weather. The disease does not develop as rapidly in cool, dry weather as it does in warm, damp weather.

He finally summarized his observations as follows—

it is much more prevalent and severe, and the incubation period is much shorter during the summer than during the winter. In Florida, the humidity and temperature are usually high during the summer, humidity averaging from 50% to 95% and temperature from 65 to 95 degrees F. at the Tropical Laboratory. Local showers are very prevalent and frequently follow one another with such rapidity that the trees do not dry off for long periods of time. During the winter, the opposite conditions prevail, the air being dry and cool and showers few with long intervals between them. At Redland, the temperature usually ranges from 45 to 85 degrees F. and the humidity from 20% to 90%. Swingle learned that the disease was much more destructive and prevalent in Japan during warm moist seasons than it was during cool dry ones.

In discussing citrus-canker in the Philippines, Mackie (*6*) states that—

during the dry season, which occurs from January until the monsoon changes in June, the disease is apparently more or less quiescent, cankers being numerous on the leaves

but not seeming to show very much on the twigs, except on the young growth and on nursery stock. However, after the rains begin, trees send out new growth and it is on this new growth the canker appears, coming into evidence in about a week. In some species, it will fairly cover the new foliage, while there also appears an abundance of canker on the twigs. Throughout the rainy season, the disease thrives, infecting practically all the young growth. This season (1917) would seem to offer ideal conditions as to climate, the weather being warm, the humidity varying from 60 to 88.

Tanaka (*16*), quoting Abe, of Japan, states that—

The severity of the organism is more pronounced in the wet years and spreads more rapidly at such times.

It can be clearly seen from the foregoing excerpts from the literature that the greatest development of canker occurs during warm, humid weather, which in some localities can be translated into the term rainy season, which in turn is usually associated with high temperatures. On the other hand, these same weather conditions stimulate the rapid growth of Citrus plants. The relation of the development of canker to the conditions of the host has been reported on by the various workers.

Stevens (*11*) says that—

young and succulent growth under humid conditions is very susceptible.

According to Wolf (*17*)—

new infections appear in spring shortly after the new growth has begun. Under favorable conditions, new infections may appear at any time throughout the growing season of the host.

Mackie (*6*), in the Philippines, says:

However, after the rains begin, trees send out new growth and it is on this new growth the cankers appear. Throughout the season, the disease thrives, infecting practically all the young growth.

Jehle (*4*, *5*) reports:

Citrus canker develops more rapidly on trees which are in a thrifty, healthy, growing condition than it does on those which are semi-dormant, unthrifty, or unhealthy. Trees in a neglected condition may harbor the disease for months before it becomes conspicuous enough to be recognized.

The vitality and vigor of the host have a marked effect upon the prevalence and severity of Citrus canker as well as upon the period of incubation. The disease is much more prevalent and severe upon trees which are in an otherwise thrifty, healthy, growing condition than it is upon those which are unthrifty and unhealthy. The period of incubation is much longer when the trees are unthrifty and unhealthy and the disease may remain on such trees in a dormant condition without becoming visible for long periods of time. . . . If a tree has become infected with the organisms, they apparently do not die, no matter how long the tree is kept in a semi-dormant or neglected condition, but persist until active growth does occur, when the canker lesions become visible.

Tanaka (*16*), quoting Bakura, of Japan, says—

it seems to attack young plants mostly.

Tanaka (*16*), quoting Nishida, of Japan, says—

I do not claim the entirely resistant nature of the Satsuma variety. It is a matter which largely depends upon the environmental condition and habit of growth of the

twigs. Satsuma does not produce as much summer growth as others, which is another reason for escaping from the severe summer infection.

All writers agree that the young and tender growth of trees in a good growing condition favors the development of the disease. Some few go so far as to give the age of the parts most susceptible. Thus, Jehle (*4*) found that—

> medium sized, thrifty leaves seem to be most susceptible, and canker is seldom found on those which are yellowish, unhealthy, very young or very old.. . . The young tender twigs and thorns are more subject to citrus canker than are the older more corky ones. . . . As the fruit matures, it seems to become less and less susceptible to citrus canker, and mature picked fruits seem to be immune.

Other investigators have also noted the absence of infection on the mature fruits.

The writer (*7*) has stated that—

> even though ideal conditions of temperature and humidity were supplied for infection, few or no canker spots developed if the plant was not in good growing condition. The largest number of spots naturally occurred on mature leaves which were still tender and of a light-green color. Few spots appeared on the young leaves, while spots developed on the old foliage of the more susceptible plants only.

The writer (*7*) has gone one step further in discussing the relations of the condition of the plant to infection when he stated that—

> apparently resistance is in part mechanical—for example, the texture of the leaf determines to a large extent the size and character of the spot. Leaf texture plays an important role in the resistance of the host plant to Citrus-canker and seems closely related to the rapidity with which the leaves mature. There is a considerable variation in the time required for the maturation of the leaves of the various Citrus plants. Thus, the leaves of the kumquat, which are rather thick and highly resistant, reach maturity much sooner than the thin, extremely susceptible leaves of the grapefruit.

Weather conditions which influence not only the growth of the organism but the trees themselves, are also responsible for retarding growth, both of the organism and the host. Thus, Jehle (*5*) finds that—

> the disease has a peculiar faculty for lying dormant for long periods without producing any visible symptoms, but sooner or later making its appearance in a typical form. There are numerous instances on record in which it has remained dormant in this way for many months on trees which have been shipped from an infected nursery.

Examples of dormancy of the organism have been encountered in the field, especially with nursery stock. The writer with Neal (*8*) proved experimentally under field conditions that the canker organisms could remain dormant through the winter in the outer bark tissue of some of the hardy hybrids for a period of 6½ months.

It is clearly evident from the facts brought out that it is extremely difficult to separate the influence of weather conditions on the development of the disease from its relations to the growth and development of the host. Even experimentally it is impossible to separate the influence of temperature and humidity. Thus, in the following experiments

the temperature was varied, but a saturated atmosphere was maintained.

Prior to placing the plants under bell jars at the various temperatures in the experiments reported on under the heading "Influence of temperature on growth of the host plants" they were thoroughly sprayed with a 48-hour-old culture of *Pseudomonas citri* in beef bouillon. All inoculations were made at 10 a. m., about which time the stomata have reached their maximum opening. As readings and observations were made on the growth of the plants notes were taken on the development of canker. Thus, a correlation could be obtained on the condition of the plant and its relation to infection and development of the disease. In Tables XI to XVII, the total number of spots and the part attacked are given. On consulting Tables VI to X it will be noted that all new spots are starred. Thus, a double check was obtained between the condition of the plant, infection, and development of the disease.

EXPERIMENT IA

On consulting Table VI it will be seen that no spots developed on any of the dormant plants of *Poncirus trifoliata*, nor on any plants subjected to temperatures below 20° C. Thus, in Table XI, only the positive results with *Citrus mitis* and *C. grandis* are included.

No spots occurred on the calamondin plants at 20° C. Canker first appeared on these plants held at 25°. At 30° the spots were more numerous, while at greenhouse temperature the number fell off. Canker was not general on these plants because they are somewhat resistant. The spots in all cases were small, unruptured, and occurred for the most part on the mature or old leaves.

Even though an extremely slow growth of grapefruit occurred at 15° C. no canker was produced. On the grapefruit plants canker first developed at 20°, the spots increasing in numbers at 25°. At 30° the number of spots dropped off considerably, while under greenhouse conditions the disease was more severe. It should be noted, however, that the grapefruit plants at 25° and those kept at the greenhouse temperature were in much better condition for infection.

TABLE XI.—*Total number of spots on plants at various temperatures*

EXPERIMENT 1A

Temperature.	*Citrus mitis.*		*Citrus grandis.*	
	Plant No. 1.	Plant No. 2.	Plant. No. 1.	Plant No. 2.
°C				
20	Clean	Clean	7 small spots on 2 mature leaves, 1 spot at tip of twig.	4 spots on leaf, 4 spots on 2 leaves.
25	4 spots on 3 old leaves	1 spot on 1 old leaf	Shoot 1: 3 spots on leaf, 31 spots on 4 leaves, 6 spots on petioles, 2 spots on twigs. Shoot 2: 4 spots on 1 mature leaf, 7 spots on leaf, 3 spots on 3 old leaves.	18 spots on 3 mature leaves.
30	6 spots on 4 old leaves	5 spots on 4 old leaves	6 spots on 5 mature leaves	1 spot on old leaf.
Greenhouse, control.	2 spots on 2 old leaves	1 spot on 1 old leaf	60 spots on 9 old leaves, 12 spots on 3 mature leaves.	1 spot on twig, 5 spots on 4 old leaves.

TABLE XII.—*Total number of spots on plants kept at 5°, 10°, and 15° C. for a period of 15 days and then transferred to 30°*

EXPERIMENT 2A, PART 1

Temperature.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*
	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.	
5° C., transferred to 30°.	Leaf 1, 1 spot at tip at midrib.	Leaf 1, 3 elongated spots along midrib.	Clean, dormant.....	Clean.....	Clean...	Clean...	Shoot 1, leaf 1, 8 spots; leaf 2, 14 spots. Shoot 2, leaf 1, 6 spots; leaf 2, 9 spots; leaf 3, 2 spots; leaf 4, 2 spots.
10° C., transferred to 30°.	Leaf 1, 6 spots at tip at midrib; leaf 2, 4 spots at tip, at midrib, 10 spots, small to elongated, on one side of new growth.	2 elongated spots at base of new growth along one side.	1 elongated spot at base of new growth.	1 spot on mature leaf, 2 elongated spots on new growth.	...do....	...do....	Leaf 1, 10 spots on petioles, 2 spots on upper side, 50 + spots on under side, 1 small spot on twig.
15° C., transferred to 30°.	Leaf 1, 1 large, loose, corky spot at base of petiole; leaf 2, 1 small spot at midrib; leaf 3, 1 small spot on petiole, at midrib.	Leaf 1, 1 large spot at petiole.	Clean, dormant.....	Leaf 1, 1 spot at base of petiole; leaf 2, 1 elongated spot at midrib; leaf 3, 1 elongated spot at midrib; leaf 4, 3 elongated spots at midrib, 2 elongated spots on new growth.	...do....	...do....	Shoot 1, leaf 1, 50 + small spots; leaf 2, 10 small spots; leaf 3, 5 small spots, 5 small spots scattering on twig. Shoot 2, leaf 1, 50 + small spots; leaf 2, 30 + small spots.

At 20° C. the spots which developed were more or less typical of those produced under natural conditions. At 25° and 30°, however, they were extremely soft, loose, and spongy. These differences were due to the stimulating influence of the high humidity and temperature.

EXPERIMENT 2A

In reality, this experiment is made up of two parts: First, the influence of temperature on infection of the plants at 5°, 10°, and 15° C., with the subsequent transfer of the bell jars, together with the plants, to the 30° case; and secondly, the infection of the plants at temperatures between 20° and 30°.

At the end of a 15-day period, the plants held at the temperatures of 5°, 10°, and 15° C. were transferred to the 30° case to see, first, if the shock would force growth of the dormant plants, and secondly, if canker would develop, for during this period no spots appeared at any of these temperatures. The appearance of new spots after the transfer is noted in Table VII, while the number and type of spots, with the part and age of the host attacked are given in detail in Table XII.

All the actively growing citranges became diseased soon after the transfer. The two plants which remained dormant stayed clean. In all cases, canker was confined to the new growth. It will be seen that most of the citrange plants developed at a normal rate after the transfer to the higher temperatures. The spots after breaking out were not scattered over the new leaves and twigs but on definite portions of the leaves, principally at the tip, along the midrib of the leaf and petiole, and, in case of twig infection, along one side in regular arrangement.

Unpublished experiments with grapefruit seedlings and plants, in both the greenhouse and field, on the time required for initial infection have shown that the organism was able to enter the leaves within 20 minutes. Apparently, when the organisms were sprayed on the plants, they were able to enter the stomata and there lie quiescent. The citrange plants were either just starting growth or were dormant when inoculated and remained so until transferred. When the plants were shifted from the 5°, 10°, and 15° C. cases to 30°, the majority of them pushed out into rapid growth, and the organisms also started to develop. As the leaves unfolded and the twigs grew in length the spots broke out where the organism had entered the tissues, which, as is stated above, occurred at definite points on the new growth. The spots appeared on the plants in from 5 to 8 days after they were placed in the 30° case.

No canker developed on the calamondin plants when they were taken from the 5° C. case and kept at a temperature of 30°. Only two plants making a rapid growth after being placed at a temperature of 30° from the 10° and 15° cases became diseased. The others remained free from canker. Both the plants which later became diseased were in a

good growing condition when first inoculated, while the others had completed their growth or were dormant. Even though some of these plants developed new growth when transferred to the higher temperature, they remained free from canker.

As on the citranges, just the new foilage was attacked in the majority of instances. The spots were present at the base of the new growth or petioles, and when present on the leaves most of them were on the midrib or near the tip of the leaves. The majority of the spots were elonagted rather than round and became visible in from five to eight days after the plants were placed in the 30° C. case.

The grapefruit plants were all in excellent condition for infection when inoculated and placed in the 5°, 10°, and 15° C. cases. However, in no instance did the disease appear at these temperatures. Immediately after the plants were transferred to the 30° case, growth proceeded at the normal rate for that temperature, and all plants showed visible spots within five days of the transfer. Canker was much more severe than on the citranges and calamondin However, the spots were limited to the young growth and were usually grouped at the tips of the young leaves. Very few spots were found scattered over the leaves in general.

Thus, while no canker occurred on any of the plants held at 5°, 10°, and 15° C. for a 15-day period, it did develop on those plants irrespective of species which were in good growing condition when inoculated, after they were all transferred to a temperature of 30°. Even though the plants did start growing after they were transferred, no canker occurred at this temperature on any which had completed their growth or were dormant when inoculated, except that one elongated spot developed at the base of the new growth on one citrange plant. Apparently, the organisms were able to enter the stomata of the very young growth and remain inactive at the lower temperatures, but when the plants were placed at a higher temperature the organisms became active and produced canker. From the location and type of the spots there is no doubt that the organism entered the tissues and remained quiescent until a higher temperature was available.

In Table XIII are given the results obtained between temperatures of 20° and 30° C. for a period of approximately four weeks. At 20° all the citrange plants became diseased. However, the spots were limited to the new growth and did not become visible until 15 days after inoculation. Only a few spots occurred on the twigs, and no mature or old leaves were attacked.

Canker was much more severe at 25° C., causing some defoliation and producing numerous spots on all plants. The spots were first visible eight days after inoculation, which is one week earlier than at 20°. The majority of the spots occurred on the young foliage. Twig canker was much more general than at 20°, and some spots were formed on the old leaves.

TABLE XIII.—*Total number of spots on plants at various temperatures*

EXPERIMENT 2 A, PART 2

Temperature.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*
	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.	
°C.							
20	Leaf 1, 3 small spots at midrib, 3 small spots at base of new growth.	Leaf 1, 6 small spots, mostly at midrib, 3 small spots at base of new growth.	Leaf 1, 1 small spot at midrib.	Clean.....	Clean.....	Clean.....	Leaf 1, upper half covered with many small corky spots; leaf 2, 7 small spots at tip of midrib.
25	Upper leaves defoliated by canker, 15 girdling spots on twig, 3 spots on 3 old leaves.	Leaf 1, 8 small spots, mostly at midrib; leaf 2, 1 small spot, 5 small spots at base of new growth, 7 small spots on old leaf below.	Leaf 1, defoliated by canker, 3 small spots at base of new growth, 5 small spots on old leaf below.	Leaf 2, 8 small spots, 3 small spots on new growth, 1 spot on old leaf.	Clean.....	Clean.....	10 spots on 2 mature leaves; growth poor.
30	Leaf 1, 11 scattering spots; leaf 2, 3 scattering spots; leaf 3, 2 scattering spots; leaf 4, scattering spots; leaf 5, scattering spots, 12 scattering spots along new growth, severe, 17 spots on 9 old leaves.	Leaf 1, defoliated by severe petiole infection; leaf 2, 4 medium spots; leaf 3, 1 medium spot and many small spots, 1 severe thorn infection, 6 small, elongated spots on new growth, 5 spots on 3 old leaves.	Leaf 1, 2 spots at petiole and midrib; leaf 2, 3 small spots, 5 medium spots on new growth.	Leaf 2, 1 small spot; leaf 3, 1 small spot, 2 small spots on twig, 6 spots on 3 old leaves.	14 spots on 4 old leaves.	20 spots on 7 old leaves.	Shoot 1, 2 leaves defoliated; leaf 3, 50 corky spots, dying. Shoot 2, leaf 1, 50 corky spots, dying, 9 spots on petiole, 2 spots on twig. Shoot 3, leaf 1, 50 spots, dying. Shoot 4, leaf 1, 25 scattering spots, severe attack.
Greenhouse	Leaf 1, 1 corky spot; leaf 2, 1 corky spot; leaf 3, 1 corky spot, 50 typical spots on 7 old leaves.	New leaves clean, 8 typical spots on 3 old leaves.	New leaves clean, 3 typical spots on 3 old leaves, 1 corky spot at base of new growth.	Clean.....	Clean.....	Leaf 1, 7 small spots; leaf 2, 1 spot at midrib.	Shoot 3, leaf 1, 2 elongated spots at midrib; leaf 2, 2 elongated spots at midrib. Shoot 4, several spots on twig. Shoot 5, leaf 1, 1 spot at petiole, 41 spots on 7 old leaves.

At 30° C., canker on old leaves and twigs was general and was much more severe on the new growth than at the lower temperatures. On one plant, the spots were visible four days after inoculation, on the others at eight days.

No consistent results were obtained at the greenhouse temperature. Very little canker occurred on the new foilage or twigs, while spots on the old leaves were common. Canker did not develop until 15 and 20 days after inoculation.

The results with the calamondin plants were rather variable. No canker occurred on these plants at 20° C. Only one rapidly growing plant inoculated at 25° became diseased, even though the other two plants made some growth later on. At 30°, canker was general on the mature and old leaves of all three plants, only two spots occurring on the new growth. One plant kept at the greenhouse temperature developed canker, and the spots here were limited to the new growth. Canker was visible 12 days after inoculation. At the other temperatures, the spots were visible in eight days. The spots produced on the calamondin plants were small and unruptured.

With the exception of the grapefruit plants kept at the greenhouse temperature, all developed canker within four days. Only two leaves were attacked at 20° C., and in both cases the spots were localized at the tip of the leaves or along the midrib. The plants held at 25° did not grow well, so that only a few spots developed on some of the mature leaves. At 30°, canker was fairly well distributed over the new foliage and twigs. Several leaves were defoliated by the severe attack, but no spots occurred on the old leaves. This is in contrast to the general distribution of canker on the plant held at the greenhouse temperature. The spots produced on the grapefruit varied with the temperature. At 20°, the spots were more typical of those found under natural conditions, while at 26° and 30° they were extremely spongy and corky. The same was true for the spots on the citranges and calamondin.

EXPERIMENT 6

In this experiment, another attempt was made to obtain infection at 15° C. There were two plants each of the trifoliate orange, Rusk citrange, calamondin, and one of grapefruit. All plants chosen were in good condition for infection. As a control a similar set was included at 20°. The plants were inoculated with a 6-day-old culture of *Pseudomonas citri* in beef bouillon, grown at 15° and 20°, respectively, set under bell jars, and kept in a saturated atmosphere for 1 month. Observations on the condition of the plants were made from time to time. It was noticed that at 15°, the young growth matured rapidly, especially that of the grapefruit plant. No spots were found at the end of the month. At 20°, on the other hand, spots were visible on the grapefruit

plant at the end of 8 days, and on the trifoliate orange and citrange plants within 20 days. One month after inoculation several tiny spots appeared on the leaves of one calamondin plant. This was the only successful infection of this species at 20° during the course of the work.

At the end of the first month, the plants held at 15° C. were transferred to the 30° case, and the set kept at 20° was abandoned. Four days after the plants were transferred to the higher temperature all were diseased, having from several to many spots. By the end of two weeks the disease was general on all the plants. The spots were more or less scattered and typical and not at all like those described in experiment 2 a. However, this was due, in part, to the fact that the leaves of the plants used in this experiment were from one-half to three-fourths grown, while the foliage of the others was mature except for the small unfolding buds. The results obtained are the some as those reported on in experiment 2 a, except that in this case the plants were held at the lower temperature 1 month instead of 15 days. Table XIV gives the total number of spots with part of the plant attacked at the temperature of 20° for one month and for two weeks after transferring the plants to the 30° case from a temperature of 15°.

EXPERIMENT 3A

According to the results of experiment 3, a varying day and night temperature had no appreciable effects on the development of the grapefruit plants. On the other hand, the effect was noticeable on the growth of the other plants used. Thus, in this experiment, canker occurred at all temperatures on the grapefruit plants, as can be seen in Table XV.

On the calamondin plants held at the constant temperature of 30° C. considerable canker developed. However, only one spot (on new growth) occurred at the varying night temperatures. In other words, the calamondin plant does not respond to so wide a temperature range for infection as grapefruit.

The citranges and the trifoliate orange plants differ from the grapefruit in their reaction to sudden changes. On the citrange, canker developed at a constant temperature of 30° C., while no spots whatever were produced on the others, in spite of the fact that they were all in the same condition when inoculated. Only a few spots occurred on a few of the trifoliate orange plants. However, the majority remained free from canker at the varying temperatures. Thus, except on grapefruit plants, a low night temperature has a tendency to inhibit infection and the development of the disease.

TABLE XIV.—*Total number of spots on plants at 20° C. and on plants at 30° after being held at 15° for one month*

EXPERIMENT 6

Temperature.	*Poncirus trifoliata.*		Rusk citrange.		*Citrus mitis.*		*Citrus grandis.*
	Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.	
20° C.....	3 small spots on 2 leaves above.	Clean.....	7 small spots on 3 leaves above, 6 small corky spots in row on twig.	Leaf 4, 19 small spots at tip; leaf 5, 2 small spots at tip.	1 small spot on leaf above.	3 small spots on 3 leaves at wound on tip.	Shoot 1, 50+ small corky spots on 1 leaf, 1 spot on twig. Shoot 2, leaf 2, 25+ small corky spots; leaf 3 25+ small corky spots; leaf 4, 25+ small corky spots. Shoot 3, leaf 1, 10 small corky spots.
15° C., transferred to 30°.	Clean.....	12 small spots scattered over upper leaves.	6 small spots on two upper leaves.	7 small spots on leaf above.	25 spots on new leaves above.	25 spots on new leaves above.	Shoot 1, 10 small spots on 2 leaves. Shoot 3, 25+ small spots on 2 leaves. Shoot 4, 30 small spots on 2 leaves.

TABLE XV.—*Total number of spots on plants held at a high temperature during the day and varying low temperatures at night*

EXPERIMENT 3A

Temperature.	*Poncirus trifoliata.*		Rusk citrange.	*Citrus mitis.*		*Citrus grandis.*	
	Plant No. 1.	Plant No. 2.		Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.
30° C., control.	1 spot on twig.	5 small spots on tip of leaf.	Shoot 1, leaf 1, 1 small spot; leaf 2, 6 small corky spots; leaf 3, 25 small corky spots; leaf 4, 5 small corky spots; leaf 6, 1 spot on petiole; leaf 7, 1 spot on petiole, defoliated, 6 spots on twig. Shoot 2, 2 spots on 2 leaves above.	Shoot 1, 1 spot on leaf above. Shoot 3, leaf leaf 1, 2 small spots; leaf 2, 5 small spots. Shoot 4, 10 small spots on twig.	Shoot 2, 20+ small spots on tip of leaf. Shoot 3, 10+ spots on tip of leaf, 1 spot on old leaf.	Shoot 1, leaf 1, 50 corky spots; leaf 2, 50+ corky spots; leaf, 3, 3 corky spots, 1 spot on old leaf. Shoot 2, leaf 1, 25+ corky spots; leaf 2, 25+ corky spots, 1 spot on twig, 2 spots on old leaf.	Leaf 1, 2 corky spots; leaf 2, 4 corky spots; leaf 3, 100+ corky spots near tip, 1 spot on twig.
20° C., transferred to 30°.	1 small spot on leaf above.	Clean.....	Clean.....	Clean.....	Clean.....	Shoot 1, 1 spot on tip of leaf, 1 corky spot on twig. Shoot 2, leaf 1, 6 small spots; leaf 2, 25 small spots; leaf 3, 50+ corky spots, 6 spots on twig.	Shoot 1, 15 small spots on twig, petiole, and midrib of tip leaf. Shoot 3, 25+ spots on twig, petiole, and midrib of tip leaf.
15° C., transferred to 30°.	Clean.....	2 spots on 2 leaves above, 1 spot on twig.	.....do.....	.....do.....	Shoot 1, 10 small spots on tip of leaves. Shoot 2, 1 spot on old leaf.	Shoot 1, 6 small spots on tip leaf. Shoot 3, 200+ spots on tip leaf, bad.	100+ small spots on leaf above, bad.
10° C., transferred to 30°.	.....do.....	Clean.....	.....do.....	.....do.....	Clean.....	Shoot 1, leaf 1, 100+ small spots, leaf 2, 5 small spots; 3 spots on twigs. Shoot 2, leaf 1, 50+ corky spots; leaf 2, 50+ corky spots; leaf 3, 25+ corky spots, 8 spots on twig.	Shoot 1, leaf 1, 25 corky spots; leaf 2, 100+ corky spots; leaf 3, 100+ corky spots, bad; leaf 4, 25+ corky spots; leaf 5, 6 small spots, 20 spots on twigs. Shoot 2, leaf 1, 11 small spots; leaf 2, 10 small spots, 6 spots on twig.

EXPERIMENT 4A

In experiment 4, it was pointed out that where plants were held for a short time at 30° C. and then placed at 15° a marked inhibition of growth occurred, although the grapefruit leaves made an extremely slow growth and the younger leaves matured to some extent. However, when transferred back to the 30° case, growth of all the plants except one proceeded at a regular rate for that temperature.

When the two sets of plants were placed in the 30° C. case, both were inoculated in the usual way. At the end of 24 hours set 2 was transferred to the 15° case to determine whether canker would develop at this temperature. No doubt the organisms were able to enter the host plants during the 24-hour interval, for canker was observed on the grapefruit plants of the control 48 hours after they were inoculated.

At 15° C. all the plants remained free from canker, with the exception of the larger grapefruit plant. Nine days after the transfer of the plants a few small, unruptured spots occurred on one grapefruit leaf (Table XVI). However, after the plants were transferred back to the 30° case, the severity of canker was as great as on the control plants, except on the one citrange plant which did not produce new growth. These results indicate quite clearly that the organisms were able to enter the plants during the interval they were held at 30° in as great a number as in the control plants, but when the plants were transferred to the 15° case, growth of the plants and likewise the development of the organism were inhibited, although in culture at this temperature a fairly good growth is made by the organism. When the plants were again placed in the 30° case and normal growth for that temperature was resumed, as much canker subsequently appeared on these as on the control plants. All experiments so far presented along this line indicate quite clearly that the development of the disease is primarily dependent upon the activity of the plant.

TABLE XVI.—*Percentage of infection on plants at an alternating high and low temperature*

EXPERIMENT 4A

Temperature.	Rusk citrange.	*Citrus mitis.*	*Citrus grandis.*	
			Plant No. 1.	Plant No. 2.
30° C.....	100 per cent leaf infection; spots few, small, and corky; 1 spot on twig at base of new growth.	Few small, scattering, compact spots on lower leaves.	100 per cent leaf infection; spots many, small to medium, corky; 2 spots at tip of 2 twigs, large and corky.	100 per cent leaf infection; spots small to medium, few corky; 1 twig spot, large and corky.
30° C., transferred to 15°.	Clean.....	Clean.....	Few small, scattering, unruptured spots on one leaf.	Clean.
15° C., transferred to 30°.	Clean; no new growth.	Spots plentiful at old leaf scars.	100 per cent leaf infection; spots many, small to large, corky; 2 twig spots at tip.	100 per cent leaf infection; spots few small to medium, corky.

EXPERIMENT 7

Heretofore, in all the experiments at low temperature no attempt was made to bring either the plants or cultures to the temperature of the case to which they were subsequently exposed. To check this phase of the work one set of plants was inoculated in the usual way. In the second set, the plants and cultures were held at 15° C. for 24 hours before the inoculations were made, to insure that both the plants and the organisms in culture were at the temperature desired. As will be noted in Table XVII, no canker developed on the plants of either set at 15° during the 18-day period they remained at this temperature. However, when both sets were transferred to the 30° case, canker appeared on the citrange and grapefruit plants in about the same proportion. The first method of inoculation which was more generally used compared favorably with the second method herein described. A similar experiment was carried out at 20°. The period of incubation, amount of infection, and growth of the plants were the same in the two experiments.

TABLE XVII.—*Comparison of methods of inoculating plants at low temperatures*

EXPERIMENT 7

Temperature.	Duration of experiment.	Rusk citrange.	*Citrus mitis.*	*Citrus grandis.*	
				Plant No. 1.	Plant No. 2.
15°–15° C.	Dec. 10 to Dec. 29, 1919.	Clean	Clean	Clean	Clean.
[a] R–15° C.	do	do	do	do	Do.
15°–15° C., transferred to 30°.	Dec. 29, 1919, to Jan. 10, 1920.	Few small spots on 1 leaf.	do	Shoot 1, leaf 1, 10 tiny spots; leaf 4, 1 small spot. Shoot 2, leaf 1, 10 small spots; leaf 2, 2 large corky spots; leaf 3, 2 small corky spots.	1 spot on 1 leaf.
R–15° C., transferred to 30°.	do	2 twig spots	do	Shoot 1, leaf 2, 5 small spots; leaf 3, 10 small spots; leaf 4, 3 small spots. Shoot 2, leaf 1, defoliated by canker; leaf 3, 1 small spot.	Bud attacked and killed by canker; no new growth.

[a] R=greenhouse temperature.

EXPERIMENT 5A

The results obtained in experiment 5 seemed to indicate clearly that at 35° C. the growth of grapefruit and plants of the same type was practically inhibited, whereas the trifoliate orange and limequat were both able to make a normal growth. It will be noted that four sets of plants were used in this experiment. After the four sets of plants remained at this temperature overnight, they were inoculated with 5-day-old cultures of the organism grown at temperatures of 10°, 15°, 25°, and 35° C., respectively.

Because of the limited amount of infection the results are not tabulated. No sign of canker developed on any of the plants in set 4, which had been inoculated with a culture of the organism grown at 35° C. As was to be expected, only three spots (two on grapefruit and one on sweet lemon) occurred on this type of plant in the other three sets. This extremely light infection was due to the distinctly inhibitive influence of the high temperature on the growth of these plants.

Many spots occurred on both the limequat and trifoliate orange plants in the remaining sets. Incubation required from 5 to 11 days on the trifoliate orange and 11 or more on the limequat plants. The spots were medium-sized, ruptured, and very corky. In no case did any of the trifoliate orange plants, which were dormant when inoculated, become infected when new growth appeared later. Furthermore, where a new shoot had started prior to inoculation, many spots developed on this shoot, but no canker appeared on any shoots which developed after inoculation. Evidently, at this temperature, the organism is unable to survive for any length of time and is only able to infect the actively growing tissue of the plant.

CONCLUSIONS ON THE INFLUENCES OF TEMPERATURE ON INFECTION AND THE DEVELOPMENT OF THE DISEASE

(1) No canker whatsoever has been produced on dormant plants.

(2) The minimum temperature for the successful inoculation of *Poncirus trifoliata*, Rusk citrange, and *Citrus grandis* plants is 20° C. Apparently, it is a little higher for plants of *C. mitis*.

(3) The optimum temperature for infection of the Citrus plants used, which were in an active growing condition, lies between 20° and 30° C., with the possible exception of *C. mitis*.

(4) A low night temperature has a decidedly inhibiting effect on infection and development of the disease on citrange and *Citrus mitis* plants. This does not hold true for *C. grandis*.

(5) At 20° C. only the new growth was attacked with few or no twig cankers; not only the new growth but twigs developed cankers at 25°, and there were few spots on old leaves; while at 30° all of these parts were readily attacked.

(6) The period of incubation varied not only with the host plant but also with the temperature. With citrange and *Citrus mitis*, the period of incubation was shortest at 30° C. With grapefruit, the period of incubation was very short at all temperatures between 20° and 30°.

(7) At 20° C. the spots produced on the plants are more typical of those found under natural conditions, while at 25° and 30° they are extremely loose, soft, and spongy.

(8) Judging from the location, parts of the plant attacked, and type of spots produced on growing plants when transferred to a temperature of 30° C. after being held from two weeks to one month at 5°, 10°, and 15° C.,

there can be no doubt that the organism entered the tissues of the host shortly after inoculation and remained quiescent until a higher temperature was available. This fact may explain the many cases of inactivity of the disease met with under field conditions.

(9) Plants held at 30° C. for 24 hours after inoculation and then transferred to a lower temperature failed to produce infection except on one grapefruit plant. However, when returned to a higher temperature, most of the plants showed 100 per cent infection.

(10) At a temperature of 35° C. infection took place only on the plants which made a normal growth, while little or no disease occurred on plants of the *Citrus grandis* type. However, all successful inoculations even on the *Poncirus trifoliata* type of plants were made with cultures of the organism grown at temperatures below 35°.

INFLUENCE OF HUMIDITY ON THE ORGANISM

The influence of humidity on bacteria resolves itself principally into a question of drying or desiccation. Bacterial growth takes place only in the presence of free moisture. Thus, in a study of the influence of humidity on bacteria, one must consider the viability of the organism and not the growth.

The common methods used heretofore have been the drying of the organisms on silk threads, glass beads, or glass slides. Some few investigators have used seeds. The method ordinarily followed by the pathologist is to smear with a sterile platinum needle on sterile microscopic slides bacteria from vigorous pure cultures and to set these slides away in the dark in a dry-air room. After a few days they are tested for viability, either by pouring nutrient agar over the slides in Petri dishes or by dropping cover glasses, which are sometimes used, into a suitable culture medium.

In the work on the resistance to drying of bacteria, no one has determined the temperature or the humidity at which the prepared slides have been kept. Again, no attention has been paid to making a uniform smear of the organism on the slides. The only factor which has been considered necessary has been that the smear be taken from young, vigorous cultures.

A brief review of the literature reveals the fact that organisms dried on seeds or on silk threads remain alive much longer than those dried on glass slides, cover glasses, or beads. However, since conditions varied with each experiment, no comparisons can be drawn.

Using the prescribed method for testing resistance to drying, Stevens (*12*) found that—

> bacteria (*P. citri*) from young and old cultures exposed for two weeks on glass slips to dry in the air of the laboratory failed to germinate.

Wolf (*17*), varying the method somewhat, states that:

The organism seems to exhibit a very considerable resistance to drying. In the desiccation experiments bacteria from vigorous pure cultures on potato plugs were smeared by means of a sterile platinum needle on clean microscopic slides in moist chambers. The moist chambers containing the microscopic slides were sterilized prior to transferring the bacterial smear to the slides. These preparations were made on June 1, and placed in a wall closet in the laboratory. On July 1, August 1, and September 1, several of the microscopic slides were removed from the moist chambers and placed in the sterilized Petri dishes, using proper aseptic precautions in making the transfers. Tubes of melted nutrient agar which had been cooled almost to the point of solidification were poured upon these smeared slides. No growth occurred in the case of those tested on September 1, but those tested on July 1 and August 1 were still alive. From this, it is believed that the organism can retain its viability for about two months.

Stevens (*12*) later carried out the following experiment:

Pieces of sterilized cloth were wetted with suspensions of bacteria (*P. citri*) from cultures of different ages, from four days old to seventy-five days old. The pieces were then allowed to dry in the air of the laboratory in the dark. Germination tests from these pieces of cloth showed a very large number of the organisms alive after a drying period of five weeks.

He also states:

That the bacteria may live for a month or more in the dried canker spots, is shown by the disease having been transferred to healthy citrus tissue from dried leaves that had been kept in the laboratory for a month.

On the other hand, Wolf (*17*) states that:

Unsuccessful attempts, however, have been made to recover the organism from the leaves kept in the laboratory from September, 1914, to May, 1915; nor has recovery been possible in the case of twig cankers kept under laboratory conditions from March to October, 1915.

Stevens (*13*) concludes from his experiments with the growth of *Pseudomonas citri* in dry sterilized soil that—

P. citri can propagate and remain alive and virulent when kept in soil for a period of twenty-six months, and that the organisms are capable of surviving long periods of desiccation without complete loss of vitality and with little apparent loss of virulence.

The following experiments, which are to be considered of a preliminary nature only, were undertaken to determine the viability of the organism at different temperatures and under various humidities.

The method used was essentially as follows: Eighteen silk threads 2 inches long were stretched across an aluminum wire frame 2⅛ inches square, with legs 1¾ inches high, inclosed in glass stockings of the same height. These frames were then placed in ordinary moist chambers 2 inches high and 3½ inches wide and sterilized in the autoclave. Larger Koch moist dishes, with ground-glass lids, were then sterilized. Under sterile conditions, the threads were immersed in a 48-hour-old culture of *Pseudomonas citri* in beef bouillon for 5 minutes. In the meantime, a

sulphuric-acid solution was added to the two dishes. The smaller dish, set in the larger one, was filled to within 1 inch of the top, and the larger dish was filled to the same height, so that about 1 inch of the smaller dish projected out from the liquids. The frames were then replaced in the smaller dishes, so that the threads were ¼ inch from the surface of the liquid. The lids of the outer dishes were then vaselined and made air-tight. At the end of each 24 hours, two silk threads were cut off and placed in tubes of beef bouillon to test for the viability of the organism. The reason for the use of two dishes, both filled with the solution, will be explained by Prof. Hottes in a forthcoming article. It is sufficient to say that this method gives a very accurate vapor pressure, which in turn could be translated into terms of relative humidity. For the sulphuric-acid concentrations, vapor pressure, and relative humidity the tables published by Stevens (*14*) were used. The specific gravity of all solutions was determined with a Twadell hydrometer when the temperature of the solution was 15° C. The dishes were set in the different temperature cases, so that they were exposed to a rather strong diffused light.

The writer wishes to point out one difficulty that had to be overcome and which caused him more or less trouble during the course of this experiment. The citrus-canker organism, as has been pointed out before, makes a very characteristic growth in beef bouillon. One of its characteristics is to produce flakes after a certain time, depending on rapidity of growth. Whenever a beef-bouillon culture of the organism which was used to inoculate the threads showed any signs of flaking, no consecutive results were obtained. Thus, several sets had to be discarded and repeated on this account. The reason is perfectly obvious and needs no further explanation. Thus, it is imperative that strictly uniform suspensions of the organism be used to inoculate the threads in order to obtain consistent results.

The results of the experiment given in Table XVIII clearly demonstrate that there is a distinct influence between temperature and humidity on the viability of the organism on the threads. At the medium humidities (49 to 70.4 per cent) the organisms were alive for the duration of the experiment at all temperatures. No organisms were viable at the end of 24 hours at the higher humidities (80.5 to 100 per cent) at 30° C. However, with each drop of 5° in the temperature more of the organisms remained viable at these humidities, until at 10° the organisms were viable at all humidities for the duration of the experiment. The same thing held true for the lower humidities. Here more or less variation existed, but there is a more or less regular sequence in the increase of viability at these humidities with each drop of 5° in temperature, until we reach 10°, where again, as is the case of the higher humidities, they are viable for eight days.

Because of the preliminary nature of this phase of the investigation no explanation of these results can be made at this time, except to point

out that there is a distinct relation between temperature and humidity on the viability of the citrus-canker organism, so far as this experiment is concerned. It is interesting to note that at the low temperatures humidity apparently has little influence on the viability of the organism on the threads, while at the higher temperatures it is the limiting factor, especially at the higher humidities. At the humidities generally encountered in the field in Alabama the organisms are viable at all temperatures on the threads for eight days. Another puzzling fact is that in ordinary distilled water the organism remained viable at temperatures between 10° and 35° C. for a period of eight days, while at the higher temperatures (30°) the organisms are dead at the end of 24 hours in a saturated atmosphere.

TABLE XVII.—*Viability of Pseudomonas citri on silk threads at varying humidities over sulphuric acid*

Temperature.	Specific gravity.	Approximate relative humidity.	Saturation deficit.	After 1 day.		After 2 days.		After 3 days.		After 4 days.		After 5 days.		After 6 days.		After 7 days.		After 8 days.	
°C.		*Per cent.*																	
30	1.00	100	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.14	89.9	3.2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.20	80.5	6.1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.25	70.4	9.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	12.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	16.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	19.5	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	1.503	18.5	25.4	+	+	+	+	−	−	−	−	+	+	−	−	+	+	+	−
	1.82	.....	.....	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
25	1.00	100.0	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.14	89.9	2.4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.20	80.5	4.6	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	1.25	70.4	7.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	9.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	12.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	14.7	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	1.503	18.5	19.4	+	+	+	+	+	+	−	−	+	+	+	+	−	+	−	−
	1.82	.....	.....	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
20	1.00	100.0	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.14	89.9	1.8	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.20	80.5	3.4	+	+	+	+	−	−	+	+	+	−	−	−	+	+	+	+
	1.25	70.4	5.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	6.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	8.9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	10.9	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	1.503	18.5	14.4	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
	1.82	.....	.....	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
15	1.00	100.0	0	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	1.14	89.9	1.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.20	80.5	2.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.25	70.4	3.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	5.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	6.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	7.9	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	1.503	18.5	10.4	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
	1.82	.....	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
10	1.00	100.0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.14	89.9	.9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.20	80.5	1.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.25	70.4	2.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	3.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	4.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	5.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.503	18.5	7.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.82	.....	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

INFLUENCE OF HUMIDITY ON GROWTH OF THE HOST PLANTS

The preliminary experiments reported below are indicative of what might be expected. Before placing the plants in the cases, all the pots were wrapped with a double layer of parraffin paper, so that no moisture could escape from the soil.

EXPERIMENT 1

Two plants each of *Poncirus trifoliata*, *Citrus mitis*, and *C. grandis* were used in each case. For the most part, the plants were dormant or had completed their growth.

Three cases with humidities of 90 to 95 per cent, 82 to 86 per cent, and 73 to 77 per cent were used. The temperature (dry bulb) in the cases varied between 21° and 23°C. As can be seen in Table XIX, with the exception of two grapefruit plants held at 90 to 95 per cent humidity, none of the plants were pushed into active growth. However, it will be remembered that at no temperature in a saturated atmosphere did the trifoliate oranges produce new growth, and likewise no results were obtained with the calamondin plants at 20° in a saturated atmosphere. The grapefruit plant did make a rapid growth at 20°, in fact much more so than those held at 90 to 95 per cent humidity and at approximately the same temperature. Thus, with dormant plants which have completed their growth, the temperature and humidities used did not stimulate the production of new growth

EXPERIMENT 2

In this experiment, three plants each of the Rusk citrange, calamondin, and grapefruit were used. One plant of each species had sufficient new growth for infection, a second had mature leaves, while the third was in a dormant condition. The results of the experiment are reported in Table XX.

Of the plants used, calamondin appeared to thrive and grow best at the humidities used in this experiment. In the experiment on the influence of temperature in a saturated atmosphere, little or no growth occurred at 20°C., but here with approximately the same temperature a good vigorous growth was made, even the dormant plants of this species starting. The results with grapefruit and citrange were not so clear-cut. Their behavior was decidedly different from that at 20° in a saturated atmosphere. Growth at the humidities used was faster, and the leaves were much smaller. Apparently, then, low humidities have the same influence as low temperatures on the maturation of the leaves of some of the Citrus plants. The cause for the decided difference in the growth of the calamondin plants is not known.

TABLE XIX.—*Growth of Citrus plants at varying humidities*

EXPERIMENT 1.

Approximate relative humidity.	Date of reading.	Number of days.	*Citrus trifoliata.*		*Citrus mitis.*		*Citrus grandis.*	
			Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.
Per cent.								
90–95	Dec. 30, 1918	0	Dormant	Dormant	Complete	Complete	Complete	Complete.
	Jan. 4, 1919	14	do	do	do	do	Starting	Starting.
	Jan. 6, 1919	16	do	do	do	do	Good	Good.
	Jan. 9, 1919	18	do	do	do	do	do	Do.
82–86	Dec. 20, 1918	0	do	do	do	do	Complete	Complete.
	Jan. 4, 1919	14	do	do	do	do	do	Do.
	Jan. 6, 1919	16	do	do	do	do	do	Do.
	Jan. 9, 1919	18	do	do	do	do	do	Do.
73–77	Dec. 20, 1918	0	do	do	do	do	do	Do.
	Jan. 4, 1919	14	do	do	do	do	do	Do.
	Jan. 6, 1919	16	do	do	do	do	do	Do.
	Jan. 9, 1919	18	do	do	do	do	do	Do.

EFFECT OF HUMIDITY ON INFECTION AND DEVELOPMENT OF THE DISEASE

The literature on this subject has already been discussed thoroughly, and the consensus of opinion has been that citrus-canker developed best and spread most rapidly in a warm, humid climate. It has also been pointed out that the host plants themselves thrive best under these influences. It has likewise been shown that the greatest number of plants are infected at 30° C. in a saturated atmosphere, while even at 20° infection takes place, particularly on grapefruit and citrange plants.

Just before the plants were placed in the humidity cases already reported on, they were thoroughly sprayed with a 48-hour-old culture of *Pseudomonas citri* in beef bouillon, which was almost allowed to dry on the foliage before they were placed in the cases.

No infections of any kind occurred on the plants listed in Table XIX during a period of 18 days.

In the second experiment (Table XX), only two infections occurred during the 15 days the plants were in the cases. Both of these occurred at the higher humidity. In one case, one spot developed on a young leaf of a calamondin plant, and several corky spots were found on the tip leaf of one grapefruit plant. No doubt, in these instances, the organism was able to enter before the plants had adjusted themselves to the humidity of the case. On January 31, 1919, the plants in both cases were removed to a saturated atmosphere and approximately the same temperature. Within eight days, one plant of the Rusk citrange, two of the calamondin, and one of the grapefruit became infected as shown in Table XXI. Only two spots on two mature leaves of one of the grapefruit plants developed on those held at the lower humidity before being transferred to a saturated atmosphere.

TABLE XX—*Growth of Citrus plants at varying humidities*

EXPERIMENT 2

Approximate relative humidity.	Date of reading.	Number of days.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*		
			Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.
Per cent.	1919.										
90–95	Jan. 16	0	Starting, 8 mm	Starting, 2 mm	Dormant	2 shoots	2 shoots	Dormant	New growth	Complete	1 shoot.
	Jan. 20	4	No change, 10 mm	No change, 4 mm	do	Good	Good	Starting	do	do	Do.
	Jan. 24	8	No change, 12 mm	No change, 8 mm	do	do	do	do	Good	do	Complete.
	Jan. 28	12	do	do	do	do	Complete	Dormant	Poor	do	Do.
	Jan. 31[a]	15	do	do	do	Good [b]	do	do	Complete [b]	do	Do.
	Feb. 5	5	Good, 15 mm	No change	do	Complete [b]	2 shoots [b]	do	do	do	Do.
	Feb. 8	8	Defoliation [b]	do	do	2 shoots [b]	Good [b]	Starting	do	do	Do.
82–86	Jan. 16	0	Starting, 4 mm	Starting	do	4 shoots	Complete	Dormant	2 shoots	do	Dormant.
	Jan. 20	4	No change	No change	do	Good	do	do	No change	do	4 shoots.
	Jan. 24	8	do	do	do	No change	do	do	do	do	No change.
	Jan. 28	12	do	do	do	do	do	do	do	do	Do.
	Jan. 31[a]	15	No change, 10 mm	No change, 1 mm	do	Good [b]	Starting	do	do	do	Do.
	Feb. 5	5	do	No change, 2 mm	do	4 shoots	Good	Starting	Poor	do	Poor.
	Feb. 8	8	do	do	do	Good	Complete	Dormant	Mature	Complete [b]	Starting.

[a] Transferred to a saturated atmosphere.

[b] New spots.

TABLE XXI.—*Total number of spots on plants held at 90 to 95 per cent and 82 to 86 per cent humidity for a period of 15 days and then transferred to a saturated atmosphere*

EXPERIMENT 2A

Approximate relative humidity.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*		
	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.
Per cent. 90–95	1 spot at base of new growth.	Clean.....	Clean.....	Shoot 1, leaf 1, 15 small spots. Shoot 2, leaf 1, 3 small spots; leaf 2, 13 small spots; leaf 3, 2 small spots.	Leaf 1, 12 small spots; leaf 2, 6 small spots.	Clean.....	Leaf 2, 23 small spots.	Clean.....	Clean.
82–86	Clean.....	...do.....	...do.....	Clean.....	Clean.....	...do.....	Clean.....	2 spots on 2 mature leaves.	Do.

CONCLUSIONS ON THE INFLUENCE OF HUMIDITY ON GROWTH OF THE ORGANISM AND HOST PLANTS AND ON INFECTION AND DEVELOPMENT OF THE DISEASE

(1) The results of the silk thread experiment prove very conclusively that there is a distinct relation between temperature and humidity on the viability of *Pseudomonas citri*.

(2) The citrus-canker organism is very susceptible to a combination of high temperature and humidity. Medium humidities at all temperatures are not injurious to the organism. With all humidities at low temperatures none of the organisms are killed.

(3) Apparently, at the humidities and temperatures used there is an inhibiting action on the growth of the Citrus plants, with the exception of *Citrus mitis*.

(4) Little or no infection occurred at the humidities and temperatures used.

DISCUSSION

The writer realizes keenly the imperfections and incompleteness of the experimental data presented, because of the complexity of the problem with its numerous and diverse factors. However, he feels that enough qualitative data have been accumulated to indicate that a most excellent field of endeavor lies in investigations of this nature. Several fundamental principles have been uncovered, which, with further study, should lead to promising results. Thus, with the incompletness of the experimental work in mind, the writer will attempt to discuss his results as a whole and correlate them with actual field conditions as he has observed them during the past four years.

A superficial study of the temperature relations, in culture, of the bacteria causing plant diseases shows that, in the main, the temperature relations of *Pseudomonas citri* agree very well with those which have been studied from time to time. One point which pathologists have not considered in their studies of temperature relations of bacteria in culture has been the time element. However, when this factor is considered, the plant-disease bacteria belonging to the Pseudomonas group have a minimum temperature of approximately 5° C. or slightly higher. By the use of former methods, lower minimums have been obtained in some cases. They have an optimum between 20° and 30°, a maximum varying with the time factor, but between 35° and 38° for a period of 24 hours, and a thermal death point between 49° and 51°. The plant-disease organisms of the bacillus group, with but few exceptions, have a maximum temperature and thermal death point several degrees lower than the Pseudomonas group.

Temperatures below the minimum simply inhibit the growth of the bacteria, so that low temperatures within reasonable limits and with the length of exposure considered do not cause their death. It should

be noted that all the active plant-disease bacteria can develop in cultures at temperatures lower than that of their host plants. The writer wishes to point out here again the pronounced lag in the growth of *Pseudomonas citri* on media between temperatures of 15° and 20° C. To him, this difference is of marked significance. No explanation of this phenomenon can be offered at this time.

In most cases, the optimum temperature for the growth of these organisms is approximately the same as that of the host plant. Thus, the temperatures at which the best development of the host plant occurs are the same as those which yield the best growth of the bacteria in culture.

For extended periods of time, the host plant develops at temperatures slightly higher than the bacteria in culture, although the plant's development is likewise retarded at the high temperatures. The extent to which the growth of the bacteria at or near the maximum is retarded or inhibited depends on the length of exposure.

While studies of the temperature relations of the bacteria in cultures are necessary, the results can not be strictly interpreted in the light of field conditions. They serve only in indicating an approximation, especially where minimum and maximum temperatures are concerned.

Our present methods of determining the resistance of bacteria to drying have been exceedingly crude, and with but few exceptions no attention has been paid to conditions which might influence the results. At best, the usual methods do not even have an empirical value, in that the results are not comparable. A glance at the literature on the subject will reveal this fact.

Different investigators have obtained widely divergent results with the same orgainsm. To illustrate, Stevens (*12*) states that—

> bacteria (*P. citri*) from young and old cultures exposed for two weeks on glass slips to dry in the air of the laboratory failed to germinate,

while Wolf (*17*) comes to the conclusion that—

> the organisms seems to exhibit a very considerable resistance to drying

and further that—

> the organism can retain its viability for about two months.

Smith (*10*)—

> found this organism (*P. campestris*) much more resistant to dry air than Harding's first report would indicate, to wit; in Harding's experiments, invariably destroyed in 45 hours, and 7 out of 8 cover-slips sterile at the end of 21 hours. In my own tests, the organism on 8 out of 24 cover-slips was alive after 34 days, when inoculated from a potato culture 2 days old and on 2 out of 23 cover-slips when inoculated from bouillon.

Later Harding, Stewart, and Prucha (*3*) found that *Pseudomonas campestris* could live on cabbage seed for a year under certain conditions.

In the experiments carried out by the writer, strict attention has been paid to the amount of the inoculum on the threads, as well as to temperature and humidity. The most striking results obtained indicate that at low temperatures humidity has little influence on the viability of the

organism, while at high temperatures it is the limiting factor. It is extremely interesting to note that at the medium humidities the organism is alive at all temperatures for the period of the experiment. Even at the extremely low humidities the organism is viable for varying lengths of time, depending somewhat on the temperature.

No attempt will be made at this time to explain the results obtained, nor to compare them with those showing that in ordinary distilled water the organism is alive at the end of eight days at temperatures between 10° and 35° C. It is sufficient to state here that the death rate of the organism on the silk threads is not due to the rapidity with which drying takes place, since at the low humidities where drying is most rapid, the death rate is slow, while at high humidities where the rate of drying is slowest the death rate is most rapid. At the medium humidities, where the organism is alive at all temperatures for the duration of the experiment, some other factor or factors must enter in other than the rapidity of drying. It should be noted that the medium humidities used in these experiments are the ones most generally prevalent under field conditions in Alabama during the greater part of the year.

The life of a plant-disease bacterium in culture in the laboratory and in the field outside of the host plant is ruled by entirely different factors from those which govern when it is parasitically active in the host tissues. Thus, a sharp distinction must be drawn between these conditions.

It is extremely difficult to compare the results obtained in the greenhouse experiments with observations in the field, because of the widely divergent conditions which exist. In the greenhouse work constant temperatures and humidity controls were used, while in the field all sorts of conditions are met. After the problem has been studied from all angles, it appears that only general statements can be made at this time.

For the purpose of this discussion, two types of rest periods can be distinguished without entering into a long explanation of the probable causes of rest periods in horticultural plants—namely, winter dormancy brought about by either the approach of cold weather or freezing temperatures and the short rest periods which occur during the growing season. During winter dormancy the cell activities cease to a great extent, while during the short rest periods which occur in the growing season some of the cell functions merely slow up.

In Alabama, as a rule, grapefruit and allied plants usually grow on into the winter, until temperatures of 5° C. or lower are reached. At this time, the plant is thrown into a state of dormancy, which persists until a period of higher temperatures occurs and active growth is resumed. This may happen several times during the winter. With Satsuma (*Citrus nobilis* var. *unshiu*, Swingle) and other mandarin oranges growth proceeds until low temperatures occur and after that no growth takes place until suitable temperatures prevail. Kumquats (*Fortunella margarita* (Lowr.) Swingle) go into dormancy and cease their growth with the

approach of low temperatures and remain dormant for a longer period in the spring than any other of the Citrus plants. The trifoliate orange, being deciduous, has a very fixed dormant period. The plants become dormant in the fall with the approach of cold weather and do not start growing until a period of favorable temperature is reached in the spring. To summarize, the Citrus plants in Alabama become dormant in the following order, trifoliate orange, kumquat, Satsuma, and grapefruit. New growth starts out in almost the reverse order, grapefruit, Satsuma, trifoliate orange, and kumquat.

Thus, with other factors eliminated, grapefruit plants develop at the lowest range of temperatures, both in the fall and spring, in the field. In all cases, the leaves formed late in the fall and early in the spring are much smaller in size and mature in a shorter period than those which are formed later in the season.

In the greenhouse experiments under control conditions it was found that grapefruit could develop very slowly at 15° C. and also that in no instance could any of the other plants used be pushed into growth at this temperature. However, at 20° all plants became active, although the calamondin, which resembles the kumquat in some respects, did not develop rapidly until a temperature of 25° was reached. The differences in the size of the leaves and time required for their maturation in comparison with those obtained at 30° were also noticed at the lower temperatures, grapefruit leaves being one-fourth to one-half the size of those produced at 30°. It was likewise observed that 16 to 20 days were required at 30° to complete the maturation of the grapefruit leaves, while at 15° 7 to 8 days were sufficient.

Thus, a mean temperature of 15° C. or thereabouts is sufficient for starting active growth of grapefruit plants in the field, while temperatures of 20° or slightly less are needed for the trifoliate orange and Satsuma. Kumquat does not start until a slightly higher mean is reached. These figures are borne out by the weather records and observations of the conditions of the plants in the field for the past four years.

The optimum temperature for the growth of the Citrus plants used in the greenhouse experiment lies between 20° and 30° C. Some differences were noted in the behavior of the different plants at these temperatures. There is no question but that at 30° the best development of all the plants occurred. Above 30° grapefruit was inhibited, while plants like the trifoliate orange seemed to make as good a growth as they did at 30°.

The short rest periods of Citrus plants during the growing season are in all probability a maturation phase, following the period of elongation of the new growth. Field observations have shown that temperature and humidity play an important part in the rate and amount of growth made during these periods; in fact, they determine to some extent the number of growth periods which occur during a season.

Because of the preliminary nature of the greenhouse experiments on the influence of humidity on Citrus plants, no statements can be made at this time, except to point out that there is a definite relation between the development of the plant and humidity.

The first prerequisite for infection of Citrus plants by *Pseudomonas citri* is the presence of free moisture on the plant. The second condition is a suitable temperature. However, with both these conditions fulfilled, no infection can take place unless the plant is in an active, growing condition. In other words, no infection of a dormant plant is possible. This fact has been clearly demonstrated by the greenhouse experiments and is borne out by observations under field conditions. During the short rest periods in summer, it is infrequent that new infections occur. This is due to the fact that the shoots have completed their growth and the period of maturation is at hand. In other words, canker is most abundant during the growth periods, the severity of the disease decreasing during the short rest period. Thus, we have cycles of infection which in turn correspond to the growth periods of the plants themselves.

In speaking of infection one must distinguish between the period of initial infection and the period of incubation. By the period of initial infection is meant the time required by the organism, after it reaches a a leaf, to enter the stomata or, in the case of wounds, the tissue of the plant. The period of incubation, on the other hand, is the period extending from initial infection until the disease is visible. As has been stated before, experiments have clearly shown that the period of initial infection is short, the organism getting into the stomata within 20 minutes. The period of incubation, on the other hand, may be short (48 hours) or long (several months), depending on external conditions.

The presence of free moisture is necessary for limited periods only in order that initial infection may take place. Initial infection does not occur at high humidities, but because of the stimulating influence of high humidities on the active growth of the plant, when accompanied by suitable temperatures, they are more conducive to the disease. As has been noted before, all investigators agree that the greatest development of canker occurs during warm, humid weather. However, in all localities where warm, humid weather prevails, we have a large rainfall. Thus, so far as initial infection and, incidentally, the development of the disease is concerned, it is not the high humidity that must be considered but the frequency of the rains. The temperature factor must not be overlooked, in that, even though frequent rains occur, no canker will develop unless a suitable temperature for the development of the organism and growth of the host is at hand. Thus, without question, even though the same amount of rain occurred in the orange districts of Japan as falls in the Gulf coast section, canker would not be so severe, because of the lower mean temperature prevailing in that country.

On the other hand, conditions are met with where a suitable temperature for growth and infection is present, but there is a decided deficiency in rainfall. The conditions existing in the Philippines can be cited as a typical example. Thus, Mackie (*6*) states that—

> during the dry season which occurs from January until the monsoon changes in June, the disease is apparently quiescent. * * * However, after the rains begin, the trees send out new growth and it is on this new growth that the canker appears, coming into evidence in about a week. * * * Throughout the rainy season, the disease thrives.

Initial infection can take place under conditions which do not favor the development of the disease. Furthermore, it may occur and the organisms may remain quiescent in the tissues for long periods of time without any signs of the disease being manifested. In fact, we may assume that there are occasions when initial infection takes place without the subsequent developent of the disease because of unfavorable conditions for its development after the organism enters the tissues of the host plant.

The writer has shown that initial infection did occur at low temperatures, although no canker developed until the plants were transferred to a higher temperature. These experiments were repeated under greenhouse conditions several times with the same results. In the field, plants were inoculated in September, 1917, and no canker became visible until the following April, when it developed very rapidly and was extremely severe on the twigs and stems of some of the hardy hybrids. No doubt, in the case of kumquat, the organism is able to enter the stomata but is unable to develop because of the resistance offered by the tissues. Where the tissues are broken, kumquat leaves can be readily infected. Thus, initial infection requires a definite set of conditions entirely different from those required for the development of the organism after it enters the host plant.

No canker whatsoever has been obtained under any conditions at 15° C. or lower on any of the plants experimented with in the greenhouse. At 20° the disease has been produced on all plants, although the amount of canker and the period of incubation varied greatly with the different plants. Thus, only one calamondin plant was successfully inoculated at 20° in all the greenhouse experiments; all growing plants became diseased at 25°; while at 30° the number of spots increased very rapidly in number over those produced at 25°.

On the trifoliate orange only a few leaf spots occurred at 20° C. after 15 days. At 25° spots were more numerous on the young leaves; a few old leaves became diseased and a few twig spots were formed. The period of incubation at this temperature was only 8 days. Canker was general on all the plants at 30°, with the period of incubation shortened to 4 days. It is interesting to note in this connection that in the eradication of canker in Alabama practically all canker on trifoliate orange

stock has been found during the months of July and August, months with the highest mean temperature. In other words, the trifoliate orange is not very susceptible at temperatures of 20°, but when temperatures of 30° are reached the period of incubation is as short as that of grapefruit, and the plants themselves are as susceptible as grapefruit, or more so. This fact can be still more clearly shown by stating that in Japan, where the temperatures are rather low and uniform during the growing season, cankers on the trifoliate orange are rare, though grapefruit and navel orange in the same orchard or nursery may be badly infected. The slow growth of the trifoliate orange, then, at temperatures around 20° makes it more or less resistant to canker, though when grown at temperatures of 30° it becomes extremely susceptible.

Grapefruit, which grows at a much lower range of temperature than any of the other Citrus plants tested, is the first plant to become infected in the spring and the last in the fall. The greenhouse experiments showed that the period of incubation at 20°, 25°, and 30° C. was 4 days. However, the spots produced at 20° were not so large or so numerous as those produced at 30°.

Thus, it has been found that the optimum temperature for the growth of the organism in culture media in the laboratory lies between 20° and 30° C. Since the same optimum has been found for the host plants, it should be expected that the same optimum should prevail for infection and development of the disease. That such is the case has been proved in the experiments reported.

At a temperature of 35° C. or thereabouts, the maximum for the growth of the organism in culture is approached, especially when the length of exposure is included. This same temperature also inhibited the growth of some plants in the greenhouse experiment. No canker was obtained on any of the plants when a culture of the organism grown at 35° was used to inoculate plants kept at this temperature in the greenhouse. Only one spot was formed on grapefruit when plants were inoculated at 35° with cultures grown at lower temperatures. The trifoliate orange appears to make a good growth at 35°, and general infections were obtained on these plants at this temperature. In the field, temperatures of 35° prevail for portions of some days over periods of several months. The question naturally arises whether the organism can exist outside the host plant for extended periods, especially if high humidities prevail at the same time. On the other hand, we know that the disease develops during these periods.

The influence of temperatures below 15° C. in the field will be discussed more fully in a forthcoming article on the overwintering of the disease. It is sufficient to state here that although a temperature of 20° is necessary for infection, the disease after it is once produced can keep on developing at temperatures lower than 20° and is fully dependent on the growth of the host plant. In other words, the canker organism is

active in the tissues so long as the host cells are active, and when the plant is forced into dormancy the organism becomes inactive and the disease is then quiescent.

From the present extent of our knowledge of this disease, it can be concluded that environmental conditions play an exceedingly important rôle in the susceptibility and resistance of Citrus plants. Thus, environmental conditions determine to some extent the anatomical structure of the plant parts attached by canker, by influencing the size and rapidity of maturation of the new growth and the leaf texture. Apparently, each species studied has a definite reaction to its environment and differs from other species in its behavior under a given set of conditions. Therefore, one should be able to forecast the susceptibility and resistance of a given plant under certain environmental conditions. Lastly, the influence of humidity and temperature on the host favors to some extent the increased or decreased virulency of the organism toward a definite species. It appears that it will be necessary to study the behavior of the host plant in its environment before any scientific selection or breeding for disease resistance can be made.

SUMMARY

(1) The temperature relations of *Pseudomonas citri* Hasse in culture are similar to those of the plant-disease bacteria of the Pseudomonas group. With the time factor included, the minimum temperature for growth in culture is about 5° C., the optimum between 20° and 30°, the maximum about 35° for a period of 24 hours, and a thermal death point between 49° and 52°.

(2) The influence of humidity on the viability of the organism is very distinct and is closely associated with temperature. At low temperatures, humidity appears to have little or no influence, while at high temperatures and high humidities it is the limiting factor. At medium humidities at all temperatures the organism is viable for the period of the experiment. Some factor or factors other than the rapidity of drying are responsible for these results.

(3) The Citrus plants used in the greenhouse experiments vary markedly in their reaction to temperatures and humidity, especially at low and high temperatures. However, with the time factor included, the optimum temperature for all the plants used lies between 20° and 30° C. With some slight variations, the same temperature relations hold in the field.

(4) Three conditions are essential for infection—the presence of free moisture on the plant, a suitable temperature, and an actively growing plant.

(5) The life of the organism in culture and outside the host plant is ruled by an entirely different set of conditions from those which

control it when it is parasitically active in the host plant. Likewise, the conditions necessary for initial infection of the plant differ.

(6) The period of initial infection must be clearly distinguished from the period of incubation and subsequent development of the disease.

(7) The conditions which bring about the most active growth of the host plant are also responsible for the most rapid development of the disease.

(8) The difference between host plants in their temperature and humidity relations, in both the greenhouse and field, is further brought out in their behavior toward infection and the development of the disease.

(9) The organism is active in the tissues so long as the host cells are active, and when the plant is forced into dormancy the organism becomes inactive and the disease is then quiescent.

(10) Environmental conditions play an exceedingly important rôle in the susceptibility and resistance of Citrus plants to canker.

(11) The results indicate that it will be necessary to study the behavior of the host plant in its environment and its relation to the causal organism before any scientific selection or breeding for disease resistance can be made.

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DAUBENTONIA LONGIFOLIA (COFFEE BEAN), A POISONOUS PLANT

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Daubentonia longifolia, known in some localities as the "coffee bean," was first brought to the attention of the Department of Agriculture when, in February, 1918, Inspector J. B. Reidy, of Houston, Tex., sent in a sample of the plant and stated that a sheepman who had lost several hundred sheep thought this plant was the cause. He reported also the result of a post-mortem examination of one of the animals.

Preliminary experiments showed that the plant is toxic, and further work has made it clear that it is very poisonous and may be the cause of considerable losses of live stock.

DESCRIPTION OF THE PLANT

Daubentonia longifolia D. C. (Pl. 62), called by some authors *Sesbania cavanillesii* Watson, is a shrub or small tree of the pulse family (Leguminosae), which includes the locusts, mesquites, etc. The leaves are alternate and pinnate, with 12 to 60 leaflets, which are oblong and pointed. The flowers, varying in color from scarlet to yellow, are in racemes which are shorter than the leaves. The pods are oblong, compressed, with four wings rising from the margins of the valves and produced beyond the sutures. The seeds are separated from one another by transverse partitions.

The plant is found on sandy soils from Florida to central Texas and as far north as the northeastern border of Texas. In some places, as in the lower Rio Grande and San Antonio regions, it is very abundant. In Houston and vicinity it is common along the roadsides and in waste places. Farther east it is confined rather closely to the Gulf region.

While this species does not appear to have been considered poisonous—in fact it is said by Havard [1] that the seeds have been used for coffee—it is an interesting fact that at various times some closely related plants have been said to be poisonous.

EXPERIMENTAL WORK

The experimental work on this plant was done in the summers of 1918 and 1919. Excluding the animals that received extracts in various forms and those which were offered the plant and refused to eat, 42 experiments were made with sheep. Table I gives a summarized statement of these experiments.

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TABLE I.—*Summary of experiments in feeding sheep with Daubentonia longifolia*

Sheep No.	Weight.	Dry weight of plant fed.	Dry weight of plant per 100 pounds of animal.	Date of feeding.	Part of plant used and method of feeding.	Effect.
	Pounds.	*Pounds.*	*Pounds.*	1918		
463	95	0.881	0.928	Sept. 25	Seed, ground and fed with balling gun	No sickness.
463	95	.823	.867	26	do	Death.
				1919		
525	80	.705	.882	June 19	do	Do.
546	95	.069	.074	July 6 to 8	Seeds, ground and fed in bran	No sickness.
517	101.5	None eaten		7 to 8	do	Do.
519		do		9 to 11	do	Do.
521	83	do		9 to 11	do	Do.
515	120	.057	.047	14 to 15	do	Do.
555	101.5	.070	.069	19 to 20	do	Do.
528	84	.370	.440	23	Seed, ground and fed with balling gun	Death.
533	105	.231	.220	25	do	Do.
550	89	.098	.110	29	do	Do.
372	128.5	.283	.022	31	do	No sickness.
372	128.5	.283	.222	Aug. 3	do	Do.
372	128.5	.283	.022	5	do	Do.
372	128.5	.283	.022	7	do	Do.
372	128.5	.283	.022	9	do	Do.
372	128.5	.283	.022	11	do	Do.
372	128.5	.283	.022	13	do	Do.
372	128.5	.283	.022	15	do	Do.
372	128.5	.283	.022	18	do	Do.
372	128.5	.283	.022	20	do	Do.
516	81.25	.033	.041	4	Pods, ground and fed with balling gun	Do.
520	100.5	.044	.044	4	do	Do.
520	100.5	.044	.044	6	do	Do.
520	100.5	.044	.044	8	do	Diarrhea, recovery.
523	89	.059	.066	6	do	No sickness.
514	142.75	.157	.011	11	do	Do.
514	142.75	.157	.011	13	do	Do.
518	128	.056	.044	11	Seed, ground and fed with balling gun	Do.
518	128	.056	.044	13	do	Do.
518	128	.056	.044	15	do	Diarrhea, recovery.
548	108	.071	.066	12	do	Slight depression, recovery.
543	90.25	.149	.165	15	Pods, ground and fed with balling gun	No sickness.
541	99	.220	.220	18	do	Do.
554	102	.281	.276	20	do	Do.
555	107	.354	.331	28	do	Do.
541	100	.441	.441	Sept. 5	do	Do.

527	89.5	.690	.722	9	Do.
544	98	.972	.992	13	Do.
548	108	.409	.379	18	Do.
526	100.5	.222	.220	20	Do.
518	127	.560	.441	22	Do.
554	107.25	1.173	1.653	22	Do.
556	110	.728	.661	26	Some discomfort.

All the experimental work was done with sheep. The cases differed somewhat in detail, but on the whole they gave a fairly good picture of the symptoms and effects. The symptoms were not so marked nor the effects so striking as in some other forms of plant poisoning.

Sheep No. 533 may be considered as a typical case. She was a ewe that had been used in another feeding experiment by which no ill effects were produced. She was in good normal condition and weighed 105 pounds at the time of the Daubentonia experiment.

On July 25, 1919, at 11.11 a. m. she was given by the balling gun 0.22 pound of ground seed per hundredweight of animal. No symptoms were noted during the day or during the next morning, but at 3.25 p. m., July 26, the pulse was rather rapid (104) and somewhat irregular. Two hours later it was still more rapid (128) and the sheep showed distinct depression. At 8.30 p. m. the pulse was 180, and the depression continued. This general condition continued with little change until 4.15 p. m., July 27, when she was down, groaning with each respiration but still able to get upon her feet. The pulse was rapid and weak. At 5.12 p. m. the sheep was down, her breathing labored, pulse imperceptible, and temperature 104.8° F. About an hour later, after no marked change, she kicked a few times and died.

The autopsy showed the heart in diastole, the lungs congested, more or less inflammation in the fourth stomach, jejunum, ileum, and cecum, the pancreas congested, and the blood vessels of the brain unusually full.

SYMPTOMS

The symptoms of Daubentonia poisoning are not very characteristic. In very light cases of poisoning little except depression is noticed. This is more marked in the severe cases. The pulse is rapid, sometimes weak and irregular, and the respiration is usually labored. The temperature in some cases was rather high, in one case being 104.8° F. This, however, would not be considered as necessarily abnormal. Diarrhea was a common symptom and may be considered as characteristic of Daubentonia poisoning. Death occurred with little or no struggling.

The experimental work showed that, in the animals which recovered, the depression and diarrhea might continue for several days. In handling sheep poisoned by Daubentonia it is important to recognize this fact and to know that recovery is likely to be a slow process.

DELAY IN PRODUCTION OF SYMPTOMS

It is somewhat difficult to determine when the first symptoms of Daubentonia poisoning are exhibited, as much depends on the acuteness of the observer in detecting changes in the behavior of the animal. Depression is the first real symptom, and it is not always easy to determine whether a sheep is slightly depressed. In determining the time elapsing between the feedings of the plant and the appearance of the first symptoms the estimate was made very conservatively, and the

actual time for toxic effects to appear was probably rather less than the figures which have been tabulated.

The time elapsing between a single feeding and the appearance of symptoms is shown in Table II.

TABLE II.—*Time elapsing between single feeding and appearance of symptoms*

Sheep No.	Dry weight of plant eaten per 100 pounds of animal.	Time before symptoms appeared.	Result.
	Pounds.	*Hours.*	
525	0.882	21	Death.
528	.440	$8^1/_5$	Do.
548	.066	$29^1/_2$	Recovery.
533	.220	$26^1/_6$	Death.
550	.110	$20^3/_4$	Do.

It is seen that the time varies from $8^1/_5$ hours to $29^1/_2$ hours, with an average of slightly more than 21 hours. Excluding sheep 528, the average would be nearly $24^1/_2$ hours. From the experimental work it appears that in most cases the symptoms appear in approximately 24 hours.

AUTOPSY FINDINGS

There was a fairly good general agreement in the pictures presented in the autopsies of the five sheep that died. The heart was generally in diastole and the lungs were congested. The fourth stomach in all cases showed more or less congestion. This was true also of the duodenum, jejunum, ileum, and cecum. Congestion in the colon was noted in only one case. The spleen and kidneys were congested, and this condition was found in the pancreas in two cases. The brain and spinal cord showed an unusual fullness of the blood vessels.

PATHOLOGICAL CHANGES IN TISSUES

In the animals poisoned, degenerative tissue changes occur principally in lymphoid tissues, smooth muscle, and in the red blood corpuscles. The more delicate cells of the lymph nodules are almost universally found to have undergone degeneration. Tissues composed of smooth muscle fibers are similarly though perhaps not so conspicuously affected. In the blood stream are many thrombi containing degenerated erythrocytes, granular material, and often fibrin.

Probably the degenerative changes in the erythrocytes and lymphoid tissues are the most important causes of the thrombus formation. Small hemorrhages due to ruptured vessels are not uncommon. Weakening of the muscle layers of the vessels, together with thrombi in the vessels, would appear to be a sufficient cause for the rupture of the vessel walls.

Degenerative changes also may occur in various glands, as the kidney and liver, but they are less severe than those in the tissues described.

TOXIC AND LETHAL DOSES

The smallest dose producing death in the experimental work was that given to sheep 550, which received 0.11 pound (49.89 gm.) per hundredweight of animal. The smallest dose producing symptoms was that given to sheep 548, 0.066 pound (29.9 gm.) per hundredweight of animal. Inasmuch as sheep 523 received 0.066 pound (29.9 gm.) per hundredweight without effect, it appears that this quantity is about the lowest limit of toxicity.

Sheep 463 is noted in Table I as receiving on September 25, 1918, 0.928 pound per hundredweight of animal without effect. However, there is no doubt that its illness on September 26, followed by death, was really the result of the feeding of September 25, for, as is shown elsewhere, the toxic symptoms ordinarily do not appear until about 24 hours after the feeding.

CUMULATIVE EFFECT

The experiments show clearly that the toxic substance of Daubentonia is excreted very slowly, so that poisoning may result from repeated administration of quantities somewhat below the toxic dosage. Sheep 520 and 518 received three doses each of 0.044 pound (19.95 gm.) per hundredweight of animal, administered on alternate days. These doses produced illness in both cases. Since the smallest single dose producing illness was 0.066 pound (29.9 gm.) per hundredweight, it is evident that there was a cumulative effect in these animals.

In this connection it should be noted that sheep 372 received on alternate days from July 31 to August 20, 0.022 pound (9.9 gm.) per hundredweight with no bad results.

COMPARATIVE TOXICITY OF PARTS OF THE PLANT

Only two experiments were made in feeding dry leaves. In sheep 556, mild symptoms were produced by 0.661 pound per hundredweight of animal. This indicated a much lower toxicity than that in the seeds.

The experimental work with extracts on guinea pigs showed that the toxicity was also present in the dry pods. The experiments of feeding pods to the sheep, however, were entirely negative, although as much as 1.653 pounds (716 gm.) per hundredweight was fed. It is evident that, as compared with the seeds, the pods are only slightly toxic and are not likely to cause any damage to live stock.

ANIMALS AFFECTED BY THE PLANT

Dr. Reidy's report was in regard to the loss of sheep, and the experimental work of the department has confirmed the toxicity of Daubentonia for these animals. Dr. Dwight H. Bennett, of the Texas Agricultural Experiment Station, has reported a case of the loss of 500 goats which were probably killed by this plant. At the present time there is no

experimental evidence of its effect on cattle and horses, but certainly it would be wise for stockmen to be very cautious about letting any domestic animals feed largely upon the fruit of the plant.

TREATMENT AND PREVENTION

No suggestions can be made for treatment other than that which would be indicated for most forms of plant poisoning. Doubtless the administration of laxatives or purgatives like linseed oil or Epsom salt would be helpful. Reliance should be placed upon prevention rather than treatment. If the plant is recognized as dangerous, stock can, with proper care, be kept from eating any considerable quantity of it. As with other poisonous plants, it is unlikely that animals eat it from choice, and they are not likely to take a quantity sufficient to produce bad results except when there is a lack of suitable forage.

So far as present knowledge goes, it appears that cases of poisoning may occur in the winter when stock, because of scarcity of other forage, are induced to eat the pods and seeds. It is at such times that animals will seize upon anything that can be eaten.

The peculiar form of the pods makes it possible for anyone to recognize the plant without difficulty, and the careful and observant stockman should be able to avoid any large losses.

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PLATE 62

Herbarium specimen of *Daubentonia longifolio*, showing flowers, leaves, and pods.



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FUSARIUM-WILT OF TOBACCO[1]

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INTRODUCTION

During the summer of 1916 the writer's attention was called to a wilt disease of tobacco occurring near Benedict, Charles Co., Md. The disease occurred on the Maryland Broadleaf variety of tobacco, which was nearing maturity, and showed all the appearances of a typical wilt disease. Plants in all stages of wilting were found, from those showing the first signs of infection to those in which all the tissues of the plant were dead. When the stalks or midribs of the leaves were cut, the fibrovascular bundles were found to have a distinctly brown to black color in place of the normal white. It was at first suspected that the bacterial wilt due to *Bacillus solanacearum* Erw. Smith had been introduced into the Maryland tobacco fields. Although the general symptoms of the disease were very similar to those of bacterial wilt, the absence of bacterial ooze, the uniform occurrence of Fusarium on plated out material, the absence of vessels filled with bacteria, and the presence of fungus strands in the vessels gave strong evidence that bacteria were not concerned. Considerable difficulty was at first encountered in getting good infection with the Fusarium isolated. When artificial infection was finally secured, however, further study of this disease became of special interest, since no Fusarium-wilt disease of tobacco has apparently been proved to exist, although, as will be shown, in one case it seemingly had been reported erroneously, and in another case a Fusarium disease, apparently not a wilt, has been described. The present paper is intended primarily to establish the occurrence of a Fusarium-wilt of tobacco, with a description of the causal organism and a discussion of certain matters bearing on the control of the disease under practical conditions.

[1] Cooperative investigations of the Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

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OCCURRENCE OF THE DISEASE

In the summer of 1916 the disease was found only on the plantation of Mr. James H. Bolling near Benedict, Charles Co., Md. It was serious in only one field of about 6 acres on this farm, where perhaps 10 to 20 per cent of the plants were dead or showed symptoms of the disease, although in smaller areas in the field it is estimated that 50 to 75 per cent of the plants were damaged (Pl. 63, A). According to Mr. Bolling and the tenant on the farm this disease had occurred at intervals for many years on this farm but not so seriously as in 1916.

During the summer of 1917 Charles County was again visted, with the result that the disease was found on two other farms near Newport, Md. The disease was not apparently so serious this season as in the previous one. This region was not visited during the seasons of 1918 and 1919, and nothing further is known of the disease in that section.

In the summer of 1919 a "new" disease of tobacco was called to the writer's attention by correspondence from Clermont Co., Ohio, and specimens were received through the courtesy of Mr. David Geesner of Owensville on September 20, which showed typical symptoms of Fusarium-wilt on mature plants of the White Burley variety. Sixty-six pieces from diseased portions were plated out, practically all of which yielded Fusarium, from which artificial infection was later secured. The disease is also said to have occurred previously in the vicinity of Owensville.

The symptoms of the disease are so evident that growers could not fail to note and report its occurrence. On account of the scarcity of such reports either from the farmers or experiment station workers in the tobacco-growing regions outside of the Granville (bacterial) wilt areas it is believed that the Fusarium-wilt is not a serious disease and probably will never become of great economic importance. If, however, it becomes more generally introduced into the White Burley districts it may become a serious parasite, since this variety, as will be shown, is very susceptible to the wilt.[1] In North and South Carolina, Georgia, and Florida where the Granville wilt occurs, it is possible that the Fusarium-wilt is also present, but growers as well as plant pathologists would be likely to report such cases as Granville wilt unless a special examination of the diseased tissue were made. It is not believed that there is much danger that this disease will become serious in the northern cigar tobacco growing regions on account of the resistance of the varieties grown and the climatic conditions prevailing.

REVIEW OF THE LITERATURE

The occurrence of Fusarium-wilt diseases of a considerable number of plants are now reported in literature. The Fusarium-wilt of tobacco possesses much in common with these diseases in that it is a vascular disease. However, it is not proposed here to enter into a review and

[1] During the summer of 1920 specimens of Fusarium-wilt were received from the White Burley district of Kentucky.

comparison of these diseases. The Fusarium problem viewed as a whole or even as that part which has to do with the nomenclature of the vascular parasites, is recognized as being in a rather unsatisfactory state. Rather uncertain precedent in naming forms, together with the plasticity in physiology, and, one is tempted to say, in morphology of the forms themselves, is the cause of the greatest difficulties encountered in this problem. It is felt, therefore, that until a more detailed study of the Fusaria causing wilt of tobacco and related plants can be made, it will not be profitable to enter upon a review preliminary to discussion of this subject. The review here presented, therefore, includes only the evidence which we now have relating to Fusarium as a probable cause of disease in the tobacco plant.

McKenney (*7*)[1] in 1903 described a wilt disease of tobacco in North Carolina as due to Fusarium. No proof of pathogenicity was obtained. This disease was soon afterward studied by Stevens and Sackett (*11*) and by Smith (*10, p. 220–271*) and was found to be a bacterial wilt (*Bacillus solanacearum*), so that Fusarium could no longer be associated with the disease. According to Smith no good evidence for a Fusarium-wilt existed; but, reasoning from the universal distribution of Fusarium and its occurrence as a vascular parasite in plants closely related to tobacco, he predicted that a Fusarium-wilt of tobacco would be found. Judging from the description of McKenney's disease and the virulence attributed to it, the writer believes it could not have been Fusarium-wilt.

Lounsbury (*6*) in 1906 reports a wilt disease of tobacco in the Kat River Valley, Cape of Good Hope, which he states is, in his opinion, not similar to the American (Granville) wilt. Bacteria, fungi, and insects are all said to be concerned. Smith (*10, p. 220–271*) places it as a doubtful bacterial wilt. To judge from the description, this may have been a Fusarium-wilt disease, at least the South African disease should again be checked up, if it still occurs.

Petch (*9*) in 1907 reported a disease of tobacco in Dumbara, Ceylon, which is said to be a "root-disease" causing "sudden and premature ripening," killing out plants in patches. The stem is said to be discolored at the base. A Fusarium was isolated from the roots. This description may fit one or more diseases of tobacco. The isolation of a Fusarium from the roots is, of course, of no significance. The "sudden and premature ripening in patches," however, suggests a wilt disease.

Delacroix (*2*) in 1906 reported a disease of tobacco occurring around Perigneux and Razoc, France, as due to a species of Fusarium which he named *Fusarium tabacivorum*. The disease is said to resemble superficially a bacterial cancer localized at the collar of the plant, and the port of entry of the parasite is believed to be always an insect puncture. The mycelium of the fungus was found to be present throughout the whole

[1] Reference is made by number (italic) to "Literature cited," p. 534–535.

base of the stalk when the disease was well established. The fungus is said to lose its virulence in culture after the "first generation." The conidia are described as straight or slightly curved, round obtuse at both extremities, possessing usually three septa, their size varying from 25 to 35 microns by 4 to 6 microns.

Delacroix's Fusarium disease is probably not a true wilt disease, since it is not described as such. The description and illustration of the causal organism are, furthermore, too fragmentary and unsatisfactory to permit of comparison. The new species created (*Fusarium tabacivorum* Delac.) has apparently not been credited by any recent workers with the Fusaria. It is interesting to note that Delacroix knew of McKenney's Fusarium disease but could not say whether his disease was identical with it or not. In view of the fact that Delacroix's description may fit other diseases of tobacco as far as symptoms are concerned, and since we have only the statement that infection has been secured with an organism of such universal occurrence as Fusarium, together with the unreliable description of the causal organism, it is difficult to see how at the present time we can accept either the disease as such or the species described as authentic.

A brief abstract was published by the writer in 1918 (*4*) calling attention to the wilt disease in Maryland and giving reasons for believing it was due to Fusarium, although artificial infection had not been secured at that time.

SYMPTOMS OF THE DISEASE

The symptoms of the disease may first become evident upon very young or on nearly mature plants. Under the field conditions observed it is evident that plants may succumb at any stage in their growth, although it is not clear as to what time the original infection of the plant occurs. It seems probable that infection may take place at any time, but that it is more likely to occur when the plants are young, the parasite remaining in a more or less latent stage until favorable environmental conditions for the further development of disease occur. In full-grown plants the earliest symptoms seem to be the sudden wilting of only one or more leaves on the plant, accompanied by yellowing and finally browning and death, but not decay of the leaf. In some cases this symptom at first may be localized on only one side of the leaf. At other times all the leaves in a narrow vertical band, comprising about one-fourth or one-eighth of the leaves of the plant, may become wilted while the others remain apparently free from the disease (Pl. 64, A). If the stalks of such plants are cut, it will be found that the discolored bundles are confined to only a part of the circumference of the vascular ring. All degrees of wilting from those described, to complete collapse of all the leaves on the plant, however, may occur (Pl. 64, B). If the plants are pulled up, large or small dead roots may be found, while others are appar-

ently healthy. If, now, the diseased stalk, roots, suckers, midribs, or veins of the leaves are cut either in cross section or longitudinally (Pl. 65, B) the vascular system will be found to be brown or distinctly black, but upon pressure no "ooze" appears. The vascular decay is distinctly "dry."

On young plants in the greenhouse where the writer has had an opportunity to note the symptoms of the disease more carefully they are essentially the same so far as the vascular system is concerned, but the leaves first lose their chlorophyll, becoming yellow and somewhat wrinkled but distinctly turgid and "brittle," as compared with healthy leaves. This condition may obtain for some time previous to wilting unless exceptionally high transpiration occurs. The leaves, of course, finally dry up as they do in the field (Pl. 63, C). In the greenhouse the symptoms are most likely to appear first on the youngest leaves, and this may be more or less characteristic in the field.

So far as has been noted the parasite is not able to cause any rotting of the living parenchymatous tissues of the plant. In heavily infested soil where the cortical layers of the plant have been severely wounded or a leaf petiole has been broken off below the surface of the soil, the parasite may enter the vascular system readily and cause the death of the aerial portion without in any way affecting the parenchyma of the stem or roots at or below the surface of the soil.

Histological studies of the disease were made by various methods, but best results were secured by killing and fixing young tissue in Gilson's fixative, imbedding in paraffin, and staining with the Pianese stain, as described by Vaughan (12). Transverse sections of infected stems or midribs of leaves (Pl. 66, A) showed that all the vessels in local areas of the vascular ring were more or less invaded, sometimes almost completely "clogged" with mycelium. Longitudinal sections (Pl. 66, B) showed in an even more striking manner the general occurrence and the "bunching" of mycelium in the vessels. Nevertheless, from the behavior of the diseased plants, especially with regard to yellowing and early turgidity, it is not believed that death of the plants is due to clogging of the vessels but rather to toxic materials formed by the parasite or as a result of the parasitic action on the host.

ISOLATION AND INFECTION EXPERIMENTS

In the first isolations pieces of the discolored portions of the stem, together with some surrounding healthy tissue, were cut out and treated with 1 to 1,000 mercuric chlorid for 30 to 120 seconds, rinsed in sterile water, and placed on hard potato agar in Petri dishes. Growth of fungus mycelium from the diseased tissue was slow and not uniform. Pure cultures of Fusarium, however, were secured. Isolations were later made by cutting off the cortical layers with a hot blade and cutting out

fairly large pieces under as sterile conditions as possible, rinsing these through 5 to 10 sterile water blanks, transferring to a sterile Petri dish, where they were further cut up into small pieces and transferred to 10 cc. of potato agar in a Petri dish acidified with two to three drops of 25 per cent lactic acid. Out of hundreds of pieces plated out in this manner apparently pure cultures of the causal organism were rapidly secured in practically all cases. Mercuric chlorid treatment apparently resulted in part of the chlorid entering the bundles, from which it was not readily washed out, and consequently did not prove useful for plating out in this case.

Single spore isolations were made from the Maryland Fusarium, and these have been used in some but not in all infection experiments, cultural studies, and spore measurements.

Infection experiments during the summer of 1917 consisted chiefly in inoculating large plants in the field with pure cultures of the Fusarium through wounds in the stalk. No infection was secured except in one instance which was questionable. In the fall of 1917 sterilized soil was inoculated with mycelium from pure cultures of the wilt Fusarium, and very young White Burley tobacco plants were transplanted into it. After about five weeks several of the plants wilted and died. Infection thereafter was intermittently secured on White Burley through the medium of the soil. The inoculum was usually grown on a mixture of 100 gm. of sand, 10 gm. of corn meal, and about 1 gm. of glucose to 50 cc. of water in 1-pint milk bottles or mason jars. This culture medium was cooked for one hour in the autoclave, then stirred up so as to render the medium "spongy," and again sterilized. After being cooled, the medium was inoculated with the Fusarium and incubated at 25° to 30° C. for four or five weeks, after which the inoculum was allowed to dry sufficiently to permit pulverizing, when it was thoroughly mixed with the soil. Good infection was also secured from mycelium and spores directly from potato agar tubes and also from a suspension of conidia alone. The latter method was usually not so successful as the former. Failure to secure as high precentage of infection at one time as at another led to a preliminary study of environmental and other conditions favoring the disease, and these will be reported upon briefly in this paper. It should be stated here, however, that as soon as the plants were intentionally wounded more uniform results in infection were secured. Ordinarily this consisted simply in pulling or pinching off one or two of the lower leaves and setting the plant deep enough to bring the resulting wound below the surface of the soil. Although it can not be said with certainty that infection would never occur in a plant perfectly free from wounds of any sort on the root or stem, it is quite certain that infection is greatly enhanced by wounding. The first signs of infection on leaves have been secured in as short a time as two days after exposing wounded stems to heavily infested soil.

CAUSAL ORGANISM

The causal organism can be readily isolated from diseased tissue by plating out on acid potato agar. The mycelium ordinarily imparts only a dull pinkish tinge to the substratum and seemingly has a more or less characteristically sparse growth and "powdery" surface (Pl. 65, A) as compared with the dense cottony development of some forms of Fusarium. The "powdery" appearance is due to microconidia which are formed in abundance, as is characteristic on a number of other media where similar growth is made. "Strains" bearing sporodochia may or may not occur. Where fruiting "strains" have been secured sporodochia have usually been produced in abundance, especially on Melilotus stems, oatmeal agar, and occasionally on potato agar and other media. True

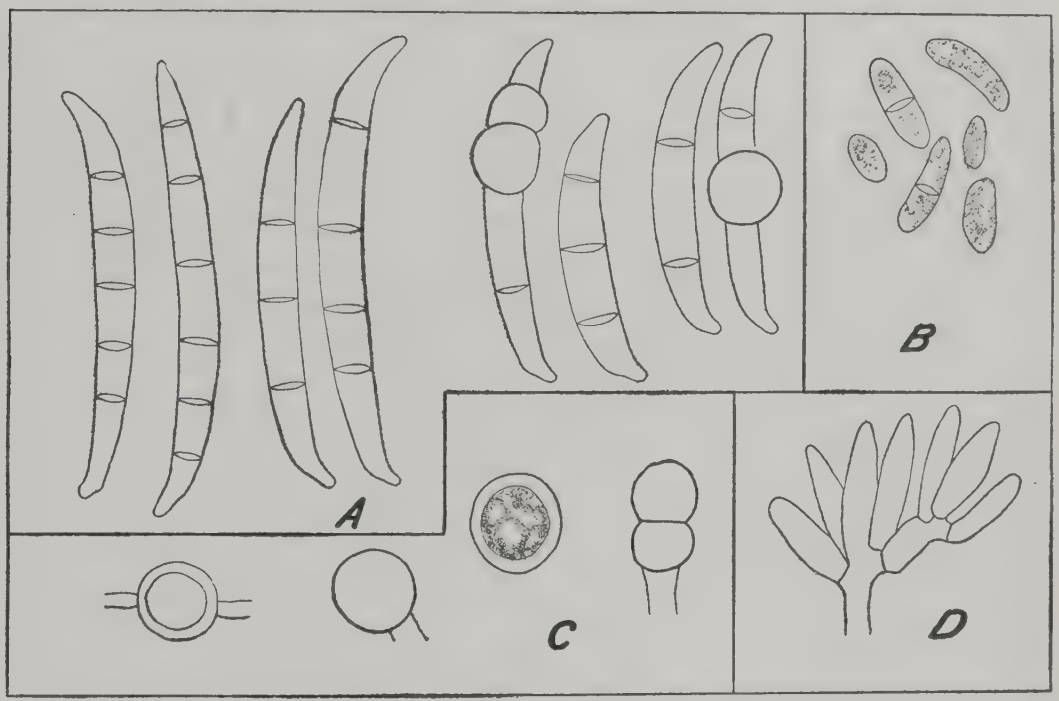


FIG. 1.—Camera-lucida drawings of spore forms of *Fusarium oxysporum* var. *nicotianae*, n. var: A, macroconidia; B, microconidia; C, chlamydospores; D, conidiophore of the sporodochial stage.

pionnotes have not been observed in the cultures during a period of four years on various kinds of media. "Pseudopionnotes" or reduced pionnotes could, however, be made to appear. Blue sclerotia and sometimes salmon-colored sclerotia are produced.

An examination of the conidia from well-developed sporodochia of the Maryland strain ordinarily shows a preponderance of 3-septate conidia, which, together with the shape and size of the spores (fig. 1) and the fact that the fungus produces a wilt disease, placed it readily in the section Elegans, according to Wollenweber's classification (*13*). A more careful study of the size and shape of the conidia brings out a close resemblance to *Fusarium oxysporum* Schlecht., according to recent descriptions of this species. Studies were therefore undertaken to establish whether the tobacco-wilt Fusarium is identical with *Fusarium oxysporum*

as described. After the conclusion was reached that the tobacco-wilt Fusarium is related morphologically to *Fusarium oxysporum* but is not identical with it, several methods of study were undertaken with the hope of furnishing further evidence. These consisted of (1) infection experiments with the tobacco-wilt Fusarium on the potato and certain other plants, (2) comparative cultural studies with *Fusarium oxysporum* strains secured from other sources, and (3) infection experiments with strains of *Fusarium oxysporum* from potato upon tobacco.

Several attempts at producing infection with the Maryland strain of the tobacco-wilt Fusarium on the potato vine failed. Potatoes were grown in artificially infested soil, and in several instances the stems were wounded immediately below the surface of the soil. This, however, is not regarded as conclusive evidence that infection is unobtainable.

Authentic cultures of *Fusarium oxysporum* were sought from various recent workers on this species. Cultures of Dr. Wollenweber's strain (No. 207) were received through Dr. W. A. Orton, and also a strain (No. 208) isolated by Dr. H. A. Edson from potatoes. From Minnesota, Bisby's culture No. 3315 and a reisolation from inoculation on potato were secured (*1*). From the stock cultures in the Department of Plant Pathology at the University of Wisconsin two strains were received, numbered 226 and 227, both apparently originally from Dr. Wollenweber to Link at Nebraska and thence to Goss at Michigan, who brought them to Wisconsin. Finally a culture of MacMillan's strain (*8*) from potatoes in Colorado, which was sent by MacMillan to the Department of Plant Pathology at Wisconsin, was obtained. None of these cultures was apparently in a good normal growing condition when transferred to my media, as compared with more recently isolated forms on the same media. The growth may be best expressed as "slimy" in nature, as if bacterial contamination had occurred—that is, aerial growth was sparse or absent and a rather thin mycelial growth was formed on the surface of the media only. Many microconidia and some macroconidia were produced. Repeated trials on various media failed to bring about the sporodochial fruiting stage, without which a satisfactory comparison with the septate conidia of the tobacco-wilt Fusarium could not be made. Therefore, the cultures were at first used largely for comparison of cultural characteristics on different media, especially on Melilotus stems, cooked rice, oatmeal agar, potato plugs, and potato agar. The various strains of *Fusarium oxysporum* from the various sources did not behave in a similar manner on the same media, and consequently it was felt that the significance of the cultural comparisons obtained was much reduced. Whether this condition was due to differences in age or condition of the strains or to actual physiological differences inherent in the strains can not be said.

The following notes were taken on the growth of the tobacco-wilt Fusarium on various media in an early trial. Not much emphasis can be

placed on the shade of the pigment given, because comparisons were not made with Ridgeway's color standards and nomenclature at this time.

ACID POTATO AGAR.—Good but rather light and "fluffy" aerial growth, pure white, no agar coloration to a pale pink coloration, and formation of blue-green sclerotial masses at margin of agar in older cultures.

POTATO PLUG.—Excellent growth, mycelium becoming faintly salmon-colored and plug deep blue in parts; abundant formation of small bluish black sclerotia in older cultures.

OATMEAL AGAR.—Good growth, pale salmon-colored mycelium, medium changing to pale lilac. Large sclerotial masses form at base of agar.

RICE.—Good growth of white to pink mycelium.

MELILOTUS STEM.—Fair growth of white to pink mycelium. Sporodochia formed abundantly after 15 to 30 days. Sporodochia forming singly or in large masses. Pale to deep salmon in color. Abundant production of small bluish black sclerotia.

STRING-BEAN PLUG.—Excellent growth with production of lilac-colored mycelium.

CARROT PLUG.—Good growth with faint lilac coloration.

LIMA-BEAN AGAR.—Fair to poor growth only, hardly any pigment production.

CORN-MEAL AGAR.—Poor growth, practically no pigment production.

SYNTHETIC AGAR.—Good growth of white mycelium.

GELATIN (BEEF).—Fair growth with some liquefaction.

TOBACCO AGAR (FROM GREEN LEAVES).—Very poor growth.

The cultural differences between the various strains of *Fusarium oxysporum* used and those of the tobacco-wilt Fusarium are not believed to be of sufficient importance to warrant presentation in detail, and only the more striking differences will be mentioned. On cooked rice the pigment of MacMillan's *F. oxysporum* was uniformly of a deeper color, appearing usually as a blue violet to jasper red as compared with light or shell pink with the tobacco-wilt Fusarium. The same was more or less characteristic on oatmeal agar, while on the other media pigment differences were insignificant. A fairly striking difference appeared with respect to the formation of sclerotial masses which came on early and in abundance on potato plugs with the tobacco-wilt Fusarium but only slowly or not at all with the *F. oxysporum* strains on hand. Sporodochia were also produced in abundance with the tobacco-wilt Fusarium on Melilotus stems but did not appear in any of the *F. oxysporum* strains, although they had, no doubt, occurred previously in these strains. In the absence of sporodochia in the cultures of *F. oxysporum* a satisfactory detailed comparison from a morphological standpoint could not, of course, be made. On the basis of certain morphological and cultural differences—that is, pigmentation and sclerotial formation, together with the failure to obtain wilt of the potato, it was at first believed that we were

dealing with a form on tobacco sufficiently distinct from *F. oxysporum* to warrant the creation of a new species. These conclusions were upset, at least for the time being, by the appearance of signs of wilt in one plant of the White Burley tobacco, out of six or eight planted, in soil inoculated with MacMillan's *F. oxysporum*. Several pots of soil were now prepared in December, 1919, and were again infested with several strains of *F. oxysporum* in comparison with my own strains secured from Maryland and Ohio, one of them being a 1916 isolation of the tobacco-wilt Fusarium which had been transferred from an old, dried culture. Good infection (about 80 per cent) was obtained with the tobacco-wilt strains and with MacMillan's strain but not with Bisby's strains (cultures in better growing condition than MacMillan's) nor with Wollenweber's strains (cultures in poorer condition than MacMillan's strain). MacMillan's strain did not, however, prove as virulent as the strains from tobacco, and the symptoms were not identical—that is, the leaves did not uniformly lose their color but presented more of a mottled appearance in the early stages of the disease, and the vascular system was not so distinctly discolored. On plating out the stem and midrib of the infested plants from MacMillan's strain in comparison with the others, the characteristic "sub-normal" condition of MacMillan's strain reappeared, showing that the strains producing the disease were the ones inoculated into the soil. A third series of inoculations was made, using all the strains of *F. oxysporum* at hand. Infection was again obtained with MacMillan's strain and with two of Wollenweber's original strains but not with the others.

In view of these results it appears that strains of *Fusarium oxysporum* may vary considerably as regards pathogenicity, but whether this is a true strain difference or merely one resulting from culturing can not be stated. It was evident, however, that the tobacco-wilt Fusarium had not suffered any loss in virulence from four years in culture, existing for a large part of this time under unfavorable cultural conditions. If *F. oxysporum* is as common in potato fields as a parasite and as common a soil saprophyte as literature would lead us to believe, it is quite surprising to us that wilt of tobacco has not been more generally noted, provided we assume the tobacco-wilt may be caused by *F. oxysporum*, since tobacco and potatoes are frequently grown in close proximity and are frequently rotated. This would be even more surprising when we add that certain varieties of tobacco are apparently more susceptible to the wilt than is the potato.

As has been stated, no infection has been secured on potato with the tobacco-wilt Fusarium, although this may sometime be accomplished. In the early work attempts were also made to get infection on tomato, cowpeas, and cabbage, but without results. Excellent infection has, however, been secured upon *Nicotiana glauca* (California tree-tobacco)

which is very dissimilar to ordinary tobacco. *N. glauca* has, in fact, been the most susceptible plant to tobacco-wilt with which we have worked. Infection has also been secured upon *N. rustica*.

On the basis of this study of the tobacco-wilt Fusarium, it is believed that although *Fusarium oxysporum* from potato is to be regarded as being able to cause a wilt of tobacco, it is not to be regarded as identical with the tobacco-wilt Fusarium as regards pathogenicity. That certain cultural differences exist has also been indicated. The final justification for placing the tobacco-wilt Fusarium as a variety of *F. oxysporum* lies in the small but nevertheless significant morphological differences which have been found to exist. These morphological differences are to be found in the somewhat larger conidia but more particularly in the higher percentages of 4- and 5-septate conidia.

One Fusarium has already been described on tobacco—*Fusarium tabacivorum*, Delac.—and although this species can not be regarded as authentic, it is thought best not to confuse the nomenclature by deriving the variety name from the specific name of tobacco. Furthermore the tobacco-wilt Fusarium is not limited to *Nicotiana tabacum* alone but attacks other members of this genus. It is therefore proposed to derive the third member of the trinomial from the generic name of tobacco. Accordingly the name *Fusarium oxysporum* (Schlecht.) Wr. var. *nicotianae*, n. var. is proposed. The following description is presented.

Fusarium oxysporum (Schlecht.) Wr. var. **nicotianae**, n. var.

Fusarium nicotianae isolated from wilting tobacco plants (*Nicotiana tabacum L.*) from Maryland and Ohio agrees quite closely morphologically with Wollenweber's diagnosis of *F. oxysporum* (Schlecht.) Wr. except in certain details not readily determined. Mycelium on most media pure white to a light pinkish tinge, of a rather "powdery" appearance, due to presence of numerous microconidia. Blue and light ochraceous salmon-colored sclerotia formed early on steamed potatoes. No true pionnotes observed. Reduced pionnotes or "pseudopionnotes" obtained. Sporodochia produced in abundance on Melilotus stems and on oatmeal agar. These are salmon-colored and when "normal" contain almost entirely 3- to 5-septate conidia, slightly larger than those of *F. oxysporum*. Three-septate conidia up to 100 per cent 34.9 by 4.2 microns (25.0 by 3.7 microns to 45.4 by 4.6 microns). Four-septate up to 40 per cent, 39.3 by 4.0 microns (29.6 by 3.7 microns to 46.3 by 4.6 microns). Five-septate up to 18 per cent 44.3 by 4.0 microns (38.9 by 3.7 microns to 51.1 by 4.1 microns). Six-septate very rare. Non-septate conidia in old sporodochia rare (7.1 by 2.4 microns). One-septate equally rare (10.1 by 2.7 microns). Two-septate up to 4 per cent (18.5 by 3.7 microns). Non-septate spores from mycelium 8.1 by 3.4 microns (10.2 by 3.7 microns to 3.7 by 2.7 microns). Chlamydospores terminal, intercalary and conidial, smooth, round, frequently in masses 8.2 microns (6 to 10.2 microns).

Pigment production not so deep as in most descriptions of *Fusarium oxysporum*.

HABITAT.—Parasitic in fibro-vascular bundles of *Nicotiana tabacum* in Maryland and Ohio, causing a decided wilting of plants followed by death. Also produces a similar disease of *N. glauca* and *N. rustica* by artificial inoculation.

CONDITIONS INFLUENCING THE DISEASE

A thorough study of the environmental conditions influencing the occurrence and extent of injury by the Fusarium-wilt disease has not been undertaken. Some evidence has been obtained, however, through experimental work and observation which is of interest in this connection. The progress of experimental work along this line was interfered with by the difficulty of obtaining a high percentage of infection even under favorable conditions, so that a considerable number of plants would have to be grown to obtain good data. This very fact should in itself stimulate further research along this line, since it is evidence that the environmental conditions most conducive to parasitism are not fully understood. Where a number of factors are involved, however, this subject becomes exceedingly complex. The introduction of such a factor as wounding, which may occur "naturally" or may vary in considerable degree when produced artificially, is a complicating factor in the tobacco-wilt disease, which in some respects renders it unfavorable for such a study.

The evidence for the conclusions presented in this paper will not be given in detail. The methods of investigation were essentially the same as those which were used in a study of the influence of soil environment on the rootrot of tobacco as described by Johnson and Hartman (5). The soil used was, however, artificially infested from pure cultures following steam sterilization. The object of the soil sterilization has been partly to secure better infestation of the soil following inoculation. In practically all cases the inoculum has been grown on 100 parts sand, 10 parts of corn meal, and 1 part of glucose to 50 parts of water. A heavy growth of mycelium and an abundance of spores on this medium undoubtedly suffice to inoculate the soil thoroughly, as is shown by instances in which "100 per cent infection" occurs (Pl. 63, B. C). Where a uniform infestation of the soil is not required, rapid infection can be secured by using conidia and mycelium from ordinary cultures placed in the soil about the wounded stems.

As will be shown later, the White Burley variety of tobacco was found to be the most susceptible to the Fusarium-wilt disease; therefore, this variety was used in all cases in the environmental studies. The use of other more resistant varieties would have rendered the securing of results far more difficult. It is assumed, however, that the same relative results would have been secured with the more resistant varieties.

The seedlings were in all cases transplanted into the infested soil from steam-sterilized soil. The root systems especially were therefore in all cases more or less wounded in their removal from the soil. Although infection has been observed in seedlings not transplanted, it is quite certain that the tobacco-wilt organism is largely dependent upon wounded

host tissue for initial infection. However this may be, it was found that wounding the plant greatly increased the possibilities of infection, and in some of the later experiments the plants were all wounded, usually by pinching or pulling off two basal leaves, in addition to the "natural" wounding resulting from transplanting.

SOIL TEMPERATURE.—Four series of experiments were run in the soil temperature control "tanks" during the winters of 1918–19 and 1919–20 in a manner similar to that which has been described for the Thielavia rootrot studies (5). Two plants in uninfested soil and two in infested soil were grown at each temperature. The temperatures usually ranged from 15° to 38° C., with intervals of 2°—that is, 12 different temperatures were used. The results in two of the trials were not convincing on account of a low percentage of infection, although the later results were approximated. In one series wilt occurred only at the approximate temperatures of 28° and 32° and not at the intermediate temperature used. In the other case, wilt occurred only at 26° to 27° and 30° to 32°. These results can only be said to indicate roughly that the higher soil temperatures are more favorable than the lower temperatures.

In the third experiment, however, more uniform infection was secured. Signs of disease were first evident at 28° to 29° and 25° to 26° C., and these were soon followed by disease at 26° to 27°, 30° to 31°, 23° to 24°, and 21° to 22°. Eighteen days later all plants in the infested soil were dead at all temperatures between 21° to 22° and 30° to 31° and also at 32° to 33°. One plant was dead and one diseased at 31° to 32°, 34° to 35°, 19° to 20°, and 17° to 18°, and two were slightly diseased at 15° to 16°. The most favorable temperature for infection and progress of the wilt appeared to be between 25° and 30°, but it seemed evident that a wide range of temperature existed within which the disease could occur.

The surface soil of the pots in the first three experiments was not insulated, though it should be, particularly in diseases of this sort where the parasite is systemic; therefore, it is quite likely that infection may have occurred near the surface where for short periods the temperature varied considerably from those given, particularly at temperatures above 30° C. Difficulties are encountered in controlling soil temperatures sufficiently accurately at all points in the soil containers in dealing with a systemic disease, though these difficulties do not play so large a rôle in cases where a parasite is limited entirely to subterranean parts. In a fourth test, using soil temperatures 3° apart, in which special attempts were made to keep the temperature at the surface of the soil constant by means of glass covers and shading of the jars in the tanks, wilt occurred first and most abundantly at 30° to 31°, and no wilting occurred at 13° to 14° or at 35° to 36°. We feel confident in concluding from the results of these experiments that the optimum temperature for the disease lies between 28° and 31°—that is, the Fusarium-wilt organism is

a warm-weather parasite, and at lower temperatures the likelihood of its occurrence is diminished. (Pl. 67, I and II.)

It is significant that the optimum for the growth of the wilt Fusarium in culture was also found to be between 28° to 30° C. Growth was very slow at 10°, and no growth was obtained at 7° and 35°. Since no growth occurred in culture at 35°, it is seemingly quite evident that no infection should occur above 35° and that chances of infection probably are considerably reduced before that temperature is reached.

It may now be recalled that the Fusarium-wilt of tobacco was first brought to our attention in 1916, when it apparently was assuming serious proportions, although it had been previously noted by the growers in lesser amounts. It will also be remembered that the summer of 1916 was one of the warmest seasons recorded by the Weather Bureau stations throughout the country, and that the soil temperature was correspondingly high that season, as shown, for instance, by records taken at depths of 2, 4, and 8 inches at Wisconsin (5). The season of 1919, when the disease occurred in Ohio, was also relatively warm. Though the evidence is scanty for the occurrence of the disease under field conditions, there is no doubt a correlation with high soil temperatures.

Soil reaction.—In experiments with Thielavia a series of soil cultures was prepared and described (5) in which the reaction of a soil of very high acidity was changed by adding varying amounts of calcium carbonate so as to give different reactions ranging from high acidity (9.38 tons lime required per acre) to one of high alkalinity. These soils have changed somewhat in reaction during the two years in which they have been used, but, as shown by the Troug color test, the same relative reaction was probably maintained. The determination of the reaction of these soils by the hydrogen-ion method, however, indicated that high alkalinity was apparently not obtained, the P_H value ranging from 5.4 to 7.2. These soils were sterilized, and one series in duplicate was inoculated with *Fusarium oxysporum* var. *nicotianae*, the other series being left as uninfested controls. Tobacco seedlings of the White Burley variety were then transplanted into them. Three separate trials were run, two of which gave reliable results, and one yielded unreliable results because of poor infection. In one experiment all the plants died at the three highest soil acidities, one died in each of the next three lower reactions, and none died in the three jars at the alkaline end, although finally they all became infected. In another experiment all plants died in the first five grades of reaction from the acid end and one in each of series 6, 8, and 9, but none in the seventh, although they were both infected. The evidence seems fairly conclusive that an acid soil favors the wilt disease, although it may occur in neutral or alkaline soils. The crocks were, however, watered from the top, and part of the soluble salts were washed downward. The soil may not have been of the same reaction throughout for this reason, but the difference could

not have been great, since the soils were repeatedly mixed and stirred. Infection apparently occurred within a wide range of soil reaction, although it was strikingly more pronounced at the higher acidities (Pl. 67, III). For this reason we can not agree with MacMillan (*8*) that infection with Fusarium is favored by alkaline soils. The behavior of Fusarium in the experiments described is also in line with the results secured in the culture of *F. oxysporum* var. *nicotianae* in culture media of varying reaction.

The Fusarium-wilt organism was inoculated in tubes of beef broth at reactions ranging from −5 per cent to +5 per cent. After 5 days the best growth was at +1. After 12 days it was apparently growing best at +3 and poorest at +5, but after about 40 days the fungus growth seemed most profuse at +5. On potato agar, however, the best growth was obtained at neutral to +0.7. After 8 days there was decidedly poorer growth as alkalinity was increased as well as a retarded growth at +1 per cent. This fungus, in common with most forms, is not favored by alkaline media, and there seems to be no good reason for expecting it to be more virulent in alkaline soils.

OTHER ENVIRONMENTAL CONDITIONS.—With respect to other environmental conditions, we are able to say very little. Observation seems to indicate that high soil moisture is not especially favorable to the disease. Infection has been noted incidentally in both relatively dry and moist soils, but the writer has been of the opinion that the soil should be kept relatively dry to get good artificial infection. The disease in Maryland occurred on high, sandy land, and the two years, 1916 and 1919, in which the disease was called to the writer's attention were both both notably hot and dry.

A single trial with soils ranging from no organic matter to pure leaf mold did not indicate any decided preference on the part of the disease for the presence of organic matter in the soil.

In the soil-inoculation experiments it has appeared that the highest infection has always been secured by planting to tobacco soon after the inoculation of the soil. Later plantings in the same soil usually resulted in a lower percentage of infection. The parasite apparently does not find the soil a very favorable medium for maintaining itself, even in the presence of host plants, and in their entire absence it probably gradually dies out completely. Nothing definite is known, however, as to how long the fungus may persist in the soil.

To summarize briefly, the conditions which seem most necessary for good infection and progress of the disease are:

1. Heavy soil infestation.
2. Wounded host tissue, particularly of stems below the surface of the soil.
3. A relatively high temperature.
4. A susceptible variety.

VARIETAL RESISTANCE TO WILT

The early infection experiments indicated that a difference in varietal resistance to the Fusarium-wilt probably existed in tobacco, but facilities for carrying out varietal tests under field conditions were not readily obtainable. It was therefore decided that preliminary tests would be carried out on a small scale, using artificially inoculated soil in greenhouse "flats" (boxes 16 inches by 24 inches and 3 inches deep). Twelve to 14 of these flats were filled with greenhouse soil and sterilized at 100° C. for two hours. When cooled, each flat was inoculated by mixing into it a sand-cornmeal culture previously referred to, after which the soil in all the flats was dumped together and again thoroughly mixed to obtain uniform infestation, and the flats were again filled. Twenty plants of each variety used were then transplanted into each flat from the sterilized soil in which they had been grown. Three series of tests were carried out, two out of doors in the summers of 1918 and 1919 and one in the greenhouse in November, 1918. In the first two tests no special attempt at artificial wounding was made. In the last series the plants were wounded by pinching off two basal leaves from each plant. Relatively higher infection was obtained in this manner. The varieties tested represent practically all the types grown commercially in the United States, and a few others, including two other species, *Nicotiana glauca* and *N. rustica*, and in one instance also an F_1 of a cross between a White Burley resistant to Thielavia rootrot and Fusarium-wilt and one susceptible to these diseases. The experiments were terminated about one month after transplanting. In taking notes on the results it was found convenient to grade the individual plants into one of four classes: 1, dead; 2, badly diseased; 3, slightly diseased; 4, healthy.

If a plant was completely wilted and dried it was classed as dead. All remaining plants showing any exterior symptoms of disease were classed as badly diseased. The remainder of the plants were then cut off close to the root system, slit longitudinally, and examined for discolored vascular systems. If any discoloration occurred attributable to infection, the plant was listed as slightly diseased, and if none occurred it was placed in the healthy class. In this manner the classification included the important conditions and yet was not wholly arbitrary. The results of the three tests are shown in Table I. In order to average these data and to arrive at a fair average figure for relative resistance expressed on the percentage basis, a more or less arbitrary formula was established. This method may be briefly described as follows: If a plant remained healthy it was credited with three points; if slightly diseased, 2 points; if badly diseased, 1 point; and if dead it was rated at zero. Twenty seedlings in one flat all healthy would be credited with 60 points (20×3), which is the maximum given and corresponds to 100 per cent resistance. Twenty seedlings in one flat all dead would receive

no credit (20×0), which is the minimum and equals 0 per cent resistance, or 100 per cent susceptibility. On the other hand, if, out of 20 plants in a flat, 5 were dead, 5 badly diseased, 5 slightly diseased, and 5 healthy, 30 points would result (5×0=0, 5×1=5, 5×2=10, 5×3=15, total 30) which is 50 per cent resistance.

It is only in some such manner, in fact, that resistance could be fairly recorded in figures. Comparative yield of plants would give no better criterion, since a plant might be infected and show no depreciation of yield and might even reach maturity and be badly diseased without appreciably influencing yield.

The average resistance given is on the basis of only 60 plants, except in a few instances when it is on a basis of only 40 or 20 plants. Though the numbers are small, they are believed to be more significant than could be obtained under field conditions with a greatly increased number of plants, because of the uniformity of the soil and of infestation.

From these calculations it will be noted that none of the varieties tried were absolutely immune. The most resistant varieties are the Connecticut Havana, Cuban, and Sumatra, with 98 per cent resistance. Since the figures are not regarded as significant within about 5 per cent, the Pennsylvania Broadleaf and the Wisconsin binder selection H12074, a strain selected for resistance to rootrot due to *Thielavia basicola*, should be included in this group. The least resistant of the *Nicotiana tabacum* varieties is the ordinary White Burley (32 per cent) (Pl. 67, IV). Strangely enough, *N. glauca*, perhaps the species farthest removed from *N. tabacum* in similarity, is the least resistant (23 per cent) to *F. oxysporum* var. *nicotianae* of all plants tried. The varieties listed have been repeatedly tried out for their resistance to the rootrot of tobacco due to *T. basicola* (*3*), and it is interesting to note the correlation in resistance to the two parasites. *N. rustica* is immune to Thielavia but may be attacked by Fusarium. Shade-grown Cuban, Little Dutch, and Wisconsin selection H12074 are very resistant to Thielavia, but, while Little Dutch is not very resistant to Fusarium, the other two are decidedly resistant. The Pryor and Oronoco types are very susceptible to Thielavia but relatively resistant to Fusarium. The White Burley, which is most susceptible to Thielavia, is also most susceptible to Fusarium. A strain of White Burley selected for resistance to Thielavia is also fairly resistant to Fusarium. The F_1 generation of a cross between resistant and susceptible Burley is seemingly intermediate in resistance to Fusarium-wilt, as it is to Thielavia. The figures for the latter are, however, not large enough to be of much significance. The cases cited seem to be sufficient to warrant the statement that the correlation between resistance in tobacco to *Thielavia basicola* and to *F. oxysporum* var. *nicotianae* is low.

TABLE I.—*Relative resistance of varieties and strains of tobacco to Fusarium-wilt*

Variety or strain.	Experiment I.					Experiment II.					Experiment III.					Average relative resistance.
	Dead.	Badly diseased.	Slightly diseased.	No disease.	Relative resistance.	Dead.	Badly diseased.	Slightly diseased.	No disease.	Relative resistance.	Dead.	Badly diseased.	Slightly diseased.	No disease.	Relative resistance.	
Connecticut Havana	0	0	2	18	97	0	0	0	20	100	0	0	1	19	98	98
Texas Sumatra											0	0	1	19	98	98
Shade-grown Cuban	0	0	0	20	100	0	1	1	18	95	0	0	0	20	100	98
Wisconsin selection H12074	0	0	2	18	97	0	0	0	20	100	0	2	2	16	90	96
Pennsylvania Broadleaf	0	1	0	19	97	0	1	0	19	97	1	1	2	16	88	94
Narrowleaf Oronoco	0	1	1	18	95	0	2	1	17	92	0	2	2	16	90	92
Blue Pryor	0	1	3	16	92	0	4	0	16	87	2	3	1	14	78	86
Connecticut Broadleaf	0	1	3	16	92	0	0	0	20	100	2	2	4	12	77	90
Little Dutch	0	3	5	12	82	1	1	2	16	88	5	7	2	6	48	73
Maryland Broadleaf	4	3	9	4	55	0	2	3	15	88	8	6	1	5	38	60
White Burley	9	3	4	4	38	9	2	4	5	41	12	5	3	0	18	32
Resistant White Burley[a]	1	2	10	7	71	0	2	3	15	88						80
F_1 (Resistant[a] × susceptible White Burley)						1	8	6	5	58						58
Nicotiana rustica						2	1	2	15	83	0	0	0	20	100	92
Nicotiana glauca						3	13	0	4	41	[b] 19	0	0	1	5	23

[a] Resistant to Thielavia rootrot.

[b] Not certain all died from wilt.

No work has been done upon the selection of resistant strains within individual varieties with the object of controlling the Fusarium-wilt disease of tobacco. From the data presented, however, it is obvious that this is a logical procedure in the control of this disease, should its economic importance warrant the undertaking. The evidence at hand indicates that the White Burley which was selected for its resistance to Thielavia rootrot also shows marked resistance to Fusarium-wilt, as compared with the ordinary White Burley, although the selection was made, of course, without reference to resistance toward Fusarium. This is, in fact, a step in the direction of control should the Fusarium-wilt become serious in the White Burley section, where, because of the susceptibility of the ordinary strains grown, it is most likely to become of economic importance. Selections in the Maryland Broadleaf variety, which is the next most susceptible of the commercial types, seems entirely feasible. Since it is on this type grown in Maryland that the disease has apparently been most common, it may be advisable in the near future to undertake to select a resistant strain of this variety unless other control measures are found which are more readily applicable.

CONTROL MEASURES

In the absence of the use of resistant varieties or strains, there appear to be only the ordinary measures of control applicable to plant parasites infesting the soil. Since the disease is due to a living organism which is carried over in the soil from year to year either as a parasite on the tobacco plant or existing as a saprophyte in the vegetable matter of the soil for possibly a limited number of years, the most evident measure of control seems to be the avoidance of infested soil. Especially when planting on new ground free from disease, it is advisable to be certain also that the seedlings to be used have not been grown on infested soil, since the parasite may be transmitted to the new soil in this manner. Using new ground for seed beds or thoroughly sterilizing old ground by means of steam is therefore desirable. New fields or seed beds receiving surface drainage water from old, infested fields should also be avoided, as should any unnecessary farm operation capable of carrying even relatively small amounts of soil from infested fields to uninfested ones. Where relatively few plants in a field are infected and show the disease, it is a good precaution to remove these plants together with the roots and to burn them so as to decrease the amount of infestation.

SUMMARY

(1) A disease of tobacco, apparently previously undescribed, has been found to occur in Maryland and Ohio. The disease is characterized by a yellowing and wilting of the leaves of the plant, usually followed by death of the entire plant. The fibro-vascular system of infected plants is characteristically brown or black.

(2) A species of Fusarium can be readily isolated from the discolored area, and infection of seedlings can be produced by inoculating the soil with this fungus. The causal organism is shown by stained paraffin sections to exist throughout the fibro-vascular system of infected parts.

(3) The Fusarium concerned seems to be closely related to *Fusarium oxysporum* (Schlecht.) Wr. but differs somewhat from this species in morphology, physiology, and pathogenicity.

(4) Infection has been secured with two strains of *Fusarium oxysporum* from potato on tobacco but has not been secured with the tobacco strain on potato.

(5) The trinomial *Fusarium oxysporum* (Schlecht.) Wr. var. *nicotianae*, n. var., is proposed for the tobacco-wilt organism.

(6) The conditions favoring infection with the tobacco-wilt organism are heavy soil infestation, wounded host tissue, a relatively high soil temperature (28° to 31° C.), and a susceptible variety.

(7) It has been found that varieties of tobacco differ markedly in their resistance to Fusarium-wilt. The White Burley variety is most susceptible, and the Havana Seed and Cuban varieties are among the most resistant.

(8) Where the disease threatens to become serious, growers are advised not to grow tobacco on the infested soils and to avoid the danger of infested seed beds. The most hopeful means of control appears to lie in the development of strains resistant to the disease within the various susceptible varieties.

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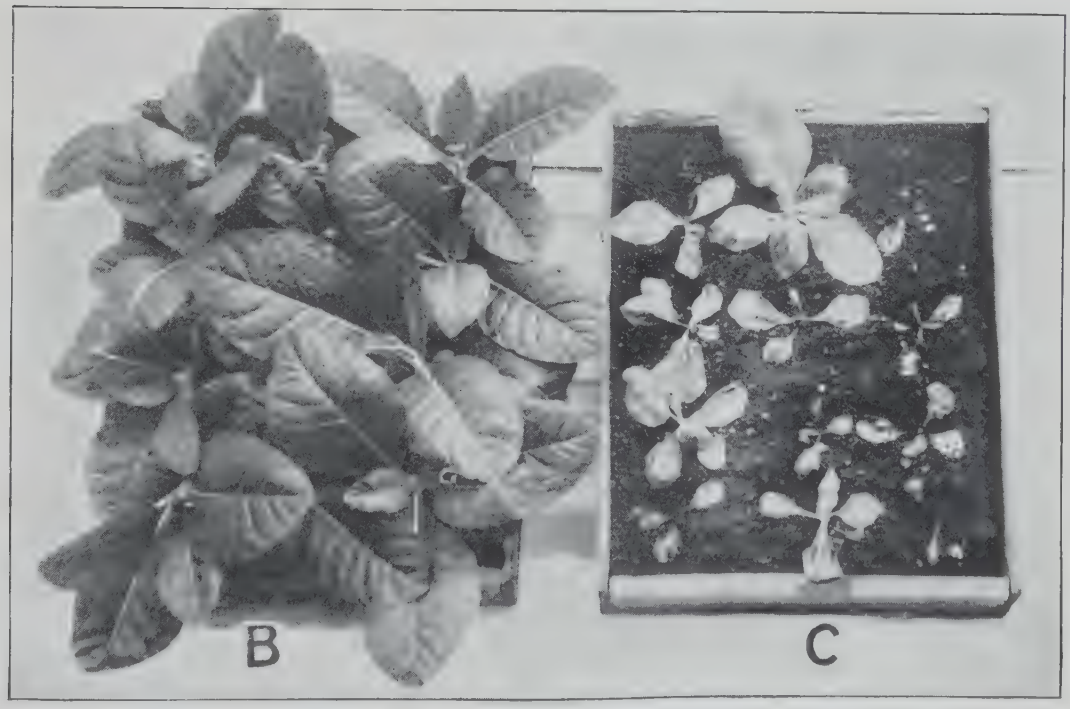
PLATE 63

A.—A typical spot in a field of Maryland Broadleaf tobacco infested with Fusarium wilt. Benedict, Md. 1916.

B.—Uninoculated control.

C.—Plants grown in soil artificially inoculated with the tobacco-wilt Fusarium and planted to White Burley.


A


B
C


B


A

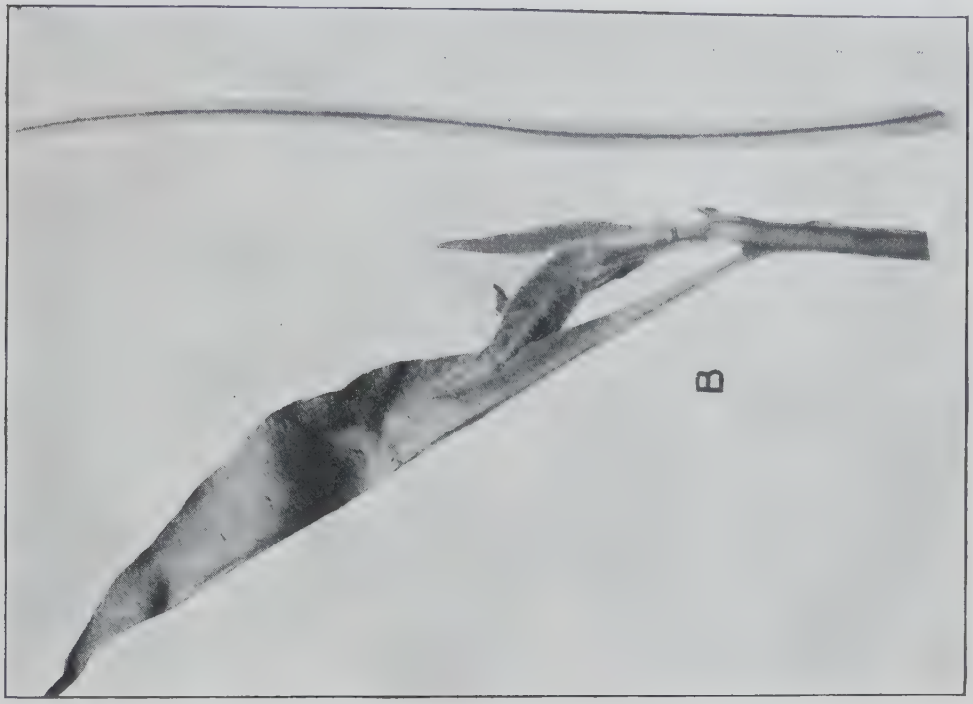
PLATE 64

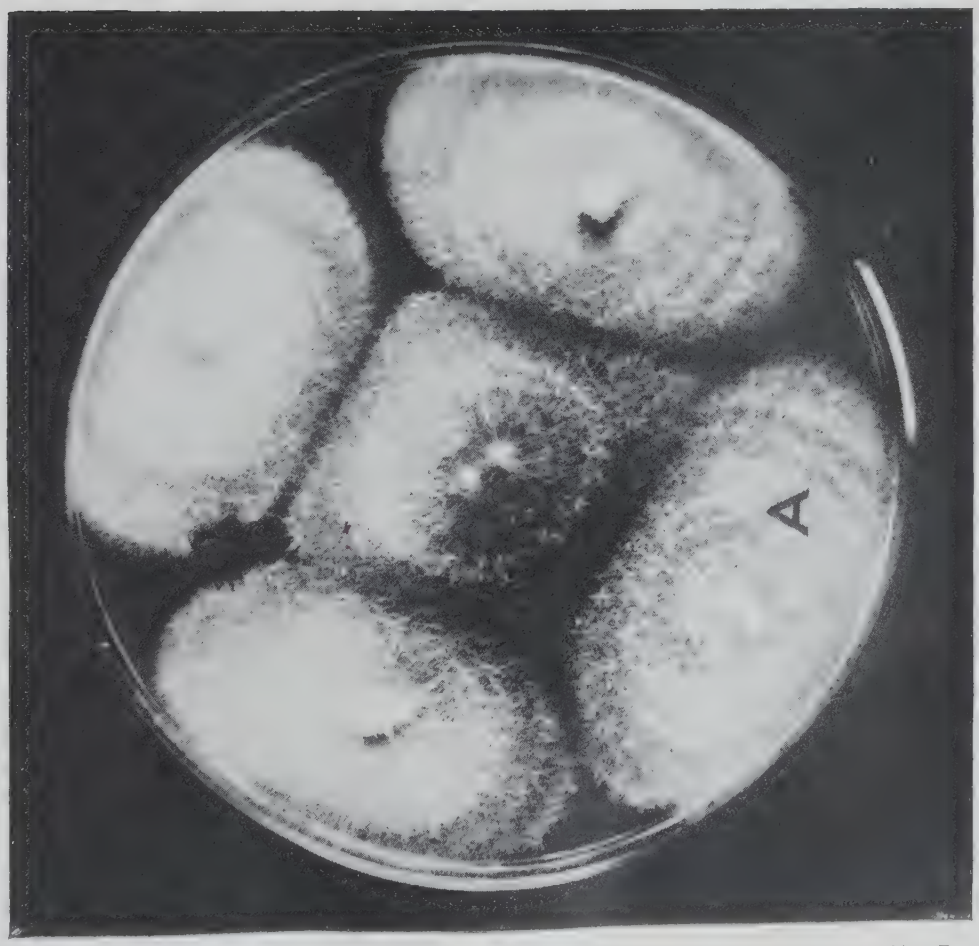
A.—Plant infected with Fusarium-wilt, showing wilting in vertical line on stalk.
B.—Last stages of Fusarium-wilt in Maryland Broadleaf tobacco.

PLATE 65

A.—Result of plating out five pieces of infected vascular tissue from infected plant, illustrating character of growth of mycelium on potato agar.

B.—Stem and midrib of plant, cut longitudinally to show the blackened vascular system.


B


A

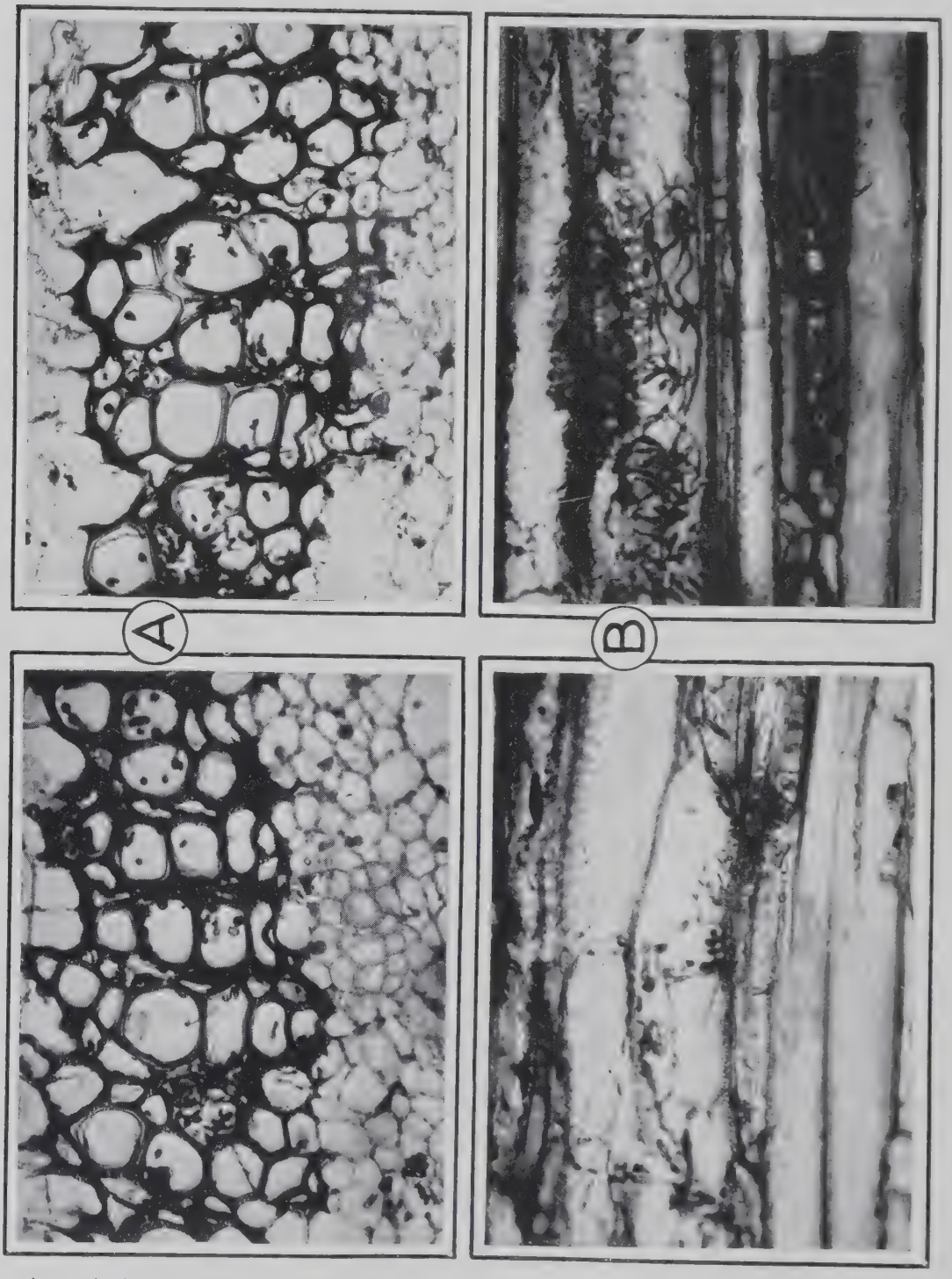

A
B

PLATE 66

A.—Cross sections through vascular system of tobacco plant infected with Fusarium-wilt, showing the fungus mycelium in the vessels. Pianese stain.

B.—Longitudinal sections through the vascular system of plants infected with Fusarium-wilt, showing the fungus strands in the vessels. Pianese stain.

PLATE 67

I.—Plants illustrating the influence of soil temperature on degree of wilting of plants in soil infested with Fusarium-wilt. The plants were grown at the following soil temperatures:

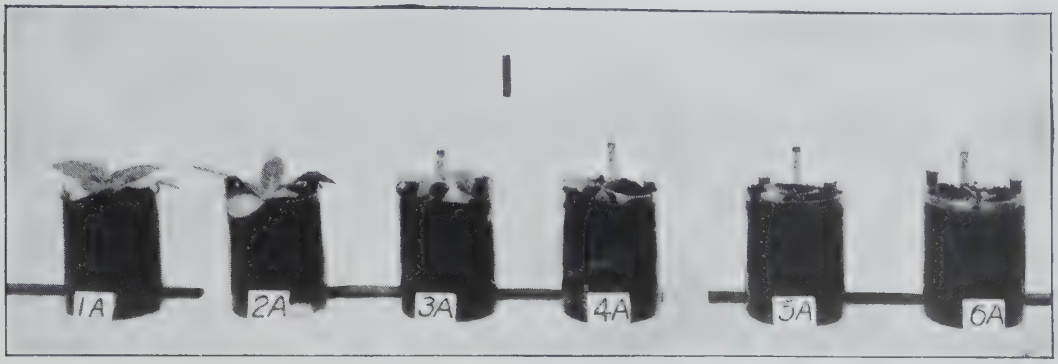
1A, 15° to 17° C.
2A, 19° to 20° C.
3A, 22° to 24° C.
4A, 26° to 28° C.
5A, 29° to 31° C.
6A, 32° to 34° C.

The upper limit for infection is close to 35°. Infection has occurred at 19° to 20°, but the progress of the disease is very slow.

II.—Plants grown in the same soil uninfested and at corresponding soil temperatures.

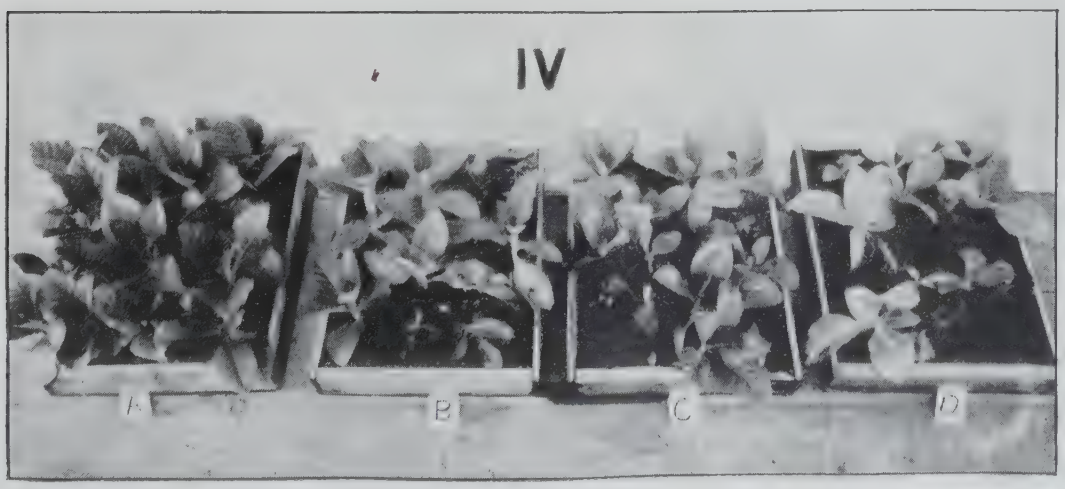
III.—Plants illustrating the influence of varying soil reaction on the amount of Fusarium-wilt in infested soil. A, highest acidity (medium to strong) to E near neutral and I alkaline end. Same soil (selected for high acidity) in all crocks but brought to various reactions by addition of precipitated calcium carbonate.

IV.—Plants illustrating varietal differences in resistance of tobacco to Fusarium-wilt. Soil artificially inoculated, uniformly mixed, and transplanted with 20 plants each of the following varieties: A, Connecticut Havana; B, Little Dutch; C, Maryland Broadleaf; D, White Burley.


I
1A
2A
3A
4A
5A
6A


II
1B
2B
3B
4B
5B
6B


III
A
B
C
D
E
F
G
H
I


IV
A
B
C
D

SUGAR BEET TOP SILAGE

By Ray E. Neidig

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The growing of sugar beets in the Pacific Northwest for the manufacture of sugar is rapidly becoming a major occupation, but the beet root from which the sugar is produced is not the only source of revenue when sugar beets are grown. There remains for the farmer a considerable portion of the crop in the form of sugar beet tops, which represent a large amount of value as a feed for stock. In recent years the farmer has utilized this source of feed, thereby securing additional revenue in the form of live stock and also in increased fertility of the soil.

It is estimated that a normal crop of sugar beets produces from 50 to 60 per cent of the weight of the crop in the form of salable beets and the remaining percentage in beet tops. This being true, it is evident that beet tops furnish no mean supply of feeding stuff for the farmer, and the careful preservation of this by-product of the beet-growing industry should be practiced.

The older countries many years ago realized the food value contained in the by-products of the sugar beet industry. Many methods have been used for the preservation of the sugar beet tops, but the siloing has received the popular choice because more food value is retained by this method than by any other. In the United States, siloing sugar beet tops has been practiced for many years. Recently the United States Department of Agriculture [2] has estmated that beet tops, when properly siloed and when fed with alfalfa hay, will reduce the hay requirement by approximately one-half. With the high prices of hay that have prevailed for the past few years, it is evident that the proper preservation of beet tops is a subject of no little economic importance.

During the past two years, numerous instances have come to the writer's notice of stock dying when fed beet top silage, and the cause of their death was attributed to the feeding of this product. However, since thousands of head of stock are successfully fed on this silage, it appeared to the writer that the fatalities were due mainly to the feeding of abnormal rather than normal silage. With the idea in mind of securing knowledge of the chemical nature of the average beet top silage as found on the average farm in the sugar beet districts, several samples of

[1] Published by the permission of Director E. J. Iddings, Idaho Agricultural Experiment Station.

[2] Jones, James W. BEET-TOP SILAGE AND OTHER BY-PRODUCTS OF THE SUGAR BEET. U. S. Dept. Agr. Farmers' Bul. 1095, 24 p., 12 fig. 1919.

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silage were collected and sent in to the chemistry department of the Idaho Agricultural Experiment Station.[1]

In the fall of 1918, four samples of beet top silage were collected from the southern part of the State by Mr. Rinehart. In 1919, six samples were collected by Mr. Aicher. All samples are representative of the average silage made in Idaho. An approximate analysis was made on each of these samples. In addition volatile and nonvolatile acid determinations were made on several of these samples of silage. The results of the approximate analysis are given in Tables I and II. Table I gives the results on the wet basis—that is, on the basis of the original moisture content—and Table II the results on the anhydrous or moisture-free basis.

TABLE I.—*Analysis of 100 gm. sugar beet top silage containing moisture*

Sample No.	Moisture.	Dry material.	Total residue left on ignition (dirt and ash).	Dirt.	Ash.	Protein.	Ether extract.	Crude fiber.	Carbohydrates (by difference).	Quality of silage.
	Per ct.	*Per ct.*	*Per cent.*	*Per ct.*	*P. ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per cent.*	
1	81.5	18.5	7.04	5.09	1.95	2.18	0.48	1.52	7.28	Poor.
2	76	24	5.29	2.32	2.97	2.95	.85	3.05	11.86	Fair.
3	59	41	17.27	12.79	4.48	4.40	.92	3.44	14.97	Poor.
4	80	20	10.51	8.42	2.09	1.88	.48	1.43	5.70	Do.
5	49.5	50.5	25.65	18.39	7.26	6.51	.68	3.89	13.77	Do.
6	68.5	31.5	11.80	7.09	4.71	4.29	1.14	2.70	11.57	Fair.
7	70	30	19.08	14.46	4.62	2.04	.53	1.91	6.44	Poor.
8	70	30	9.79	5.13	4.66	4.38	.84	2.60	12.39	Fair.
9	78.2	21.8	14.13	11.65	2.48	1.38	.26	1.10	4.93	Poor.
10	74.4	25.6	12.22	8.26	3.96	2.71	.29	2.00	8.38	Do.

TABLE II.—*Analysis of 100 gm. moisture-free sugar beet top silage*

Sample No.	Total residue left on ignition (dirt and ash).	Dirt.	Ash.	Protein.	Ether extract.	Crude fiber.	Carbohydrates (by difference).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	38.04	27.51	10.53	11.80	2.57	8.22	39.37
2	22.04	9.65	12.39	12.28	3.54	12.69	49.45
3	42.12	31.20	10.90	10.74	2.24	8.38	36.51
4	52.55	42.10	10.45	9.40	2.40	7.15	28.50
5	50.81	36.41	14.37	12.89	1.35	7.70	27.26
6	37.45	22.50	14.95	13.61	3.62	8.57	36.75
7	63.59	48.19	15.38	6.79	1.76	6.36	21.50
8	32.60	17.08	15.52	14.59	2.80	8.66	41.35
9	64.82	53.44	11.38	6.35	1.17	5.17	22.49
10	47.73	32.26	15.47	10.58	1.12	7.81	32.76

An examination of the results shows that only three of the samples were classed as fair silage. The remaining seven samples were classed as beet top silage of poor quality. A noteworthy fact seen from the inspec-

[1] The collecting of the samples was made possible through the kind cooperation of Mr. E. F. Rinehart, Field Animal Husbandryman for Idaho, and Superintendant L. C. Aicher, of the Aberdeen substation. The writer wishes to thank these men for their careful notations of general conditions and their interest and cooperation in the work.

tion is the high percentage of dirt or sand found in the residue after ashing. The real or true ash of the beet top silage was separated from the total residue after igniting in an electric furnace, the difference representing sand or dirt. Even on the basis of the silage containing the original moisture it is seen that the percentage of dirt is high in the three samples classed as fair silage, the amount ranging from 2.32 pounds to 7.09 pounds on the basis of the wet silage. When calculated on the moisture-free basis these samples contain dirt and sand to the amounts of 9.65 and 17.1 pounds per 100 pounds of moisture-free silage. On the other hand, the amount of dirt found in the poorer grades of silage ranges on the wet basis from 8.26 pounds to 18.39 pounds per 100 pounds of wet silage, while on the basis of 100 pounds moisture-free silage there are found from 22.50 to 53.44 pounds. These figures are all the more striking when applied to the average daily amount of beet top silage eaten by stock. An animal consuming an average ration containing 35 pounds of beet top silage must necessarily consume from 2.89 pounds to 6.44 pounds of dirt. It is not unfair to assume that such quantities of dirt, which in most localities engaged in growing sugar beets is a light, sandy, volcanic ash, would tend to produce serious digestive disturbances which in turn might produce the death of the animal. In samples 4 and 9, death of stock did actually take place while the silage was being fed. An inspection of the dirt content of these two silages shows a dirt content of 8.42 and 11.65 pounds in every 100 pounds of wet silage and 42.1 and 53.44 pounds, respectively, in every 100 pounds of moisture-free silage.

The reasons for the presence of such a large quantity of dirt in the silage are many. A brief summary of the methods used by the average farmer when siloing sugar beet tops will be given, since it will tend to explain the large quantities of sand and dirt that are present. In the first place, the type of silo is very crude. Usually it is a shallow dirt trench or pit of sufficient size to accommodate the crop of beet tops. The beet tops are thrown into piles in the field and scooped upon wagons. More or less dirt clings to the beet tops, especially if this work is carried on in rainy weather. The wagons are driven into the trench and dumped, each load tending to pack the beet tops previously unloaded. Such procedure does not hinder but rather aids in the carrying in of some dirt. It is readily seen that the whole process of siloing sugar beet tops is one where dirt is collected in all steps of the process from the time of topping the beets until the tops are actually siloed, unless extreme care is used to keep out excess dirt. Without extreme care a good silage can not be obtained. The United States Department of Agriculture has recently issued a bulletin [1] which sets forth the best methods of siloing sugar beet tops and describes the best types of pit silos. Pit silos with concrete side are recommended. Many good suggestions as to the proper

[1] JONES, James W. OP. CIT.

methods that a farmer should use are given. From the study of Tables I and II of this paper it is plain that more care is needed on the part of the average farmer before he can expect to secure a silage of good quality. If the suggestions embodied in farmer's bulletins of the United States Department of Agriculture are followed, the farmer will not only be rewarded with a silage of good quality and high feeding value but he will also avoid the loss of stock.

ACIDITY OF SUGAR BEET TOP SILAGE

Investigations of many types of silage by the writer [1] and others have indicated that in practically all silages that have undergone a normal fermentation there results an acidity in which the chief acids are lactic, acetic, and propionic, their relative importance decreasing in the order named. In the sugar beet top silage it was desired to study the acidity of several samples to learn what types of acids were formed in the silage found on the average farm. With this idea in mind, several of the samples sent in to the experiment station were examined. The Duclaux method [2] was used for estimating the volatile acids, and the zinc lactate method was used for the nonvolatile or lactic acid. The algebraic and graphic methods described by Gillespie and Walters [3] were used in calculating the individual volatile acid after they were identified by the qualitative tests suggested by Dyer.[4] The results on the volatile and nonvolatile acids are given in Tables III and IV. Table III gives the results on the wet basis and Table IV gives the results on the moisture-free basis.

An inspection of Tables III and IV shows that the acids developed in the sample of sugar beet top silage are not similar to those usually found in the corn silage. Corn silage contains lactic, acetic, and propionic acids. The proportion of lactic to the two volatile acids is usually about 1 part to 75 hundredths, while the proportion of acetic to propionic is usually 1 part to one-tenth. Butyric acid was never found in silage that was classed as good corn silage. It was found, however, in partially spoiled samples. Hence the conclusion was reached that silage containing butyric acid has undergone an abnormal fermentation.

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[1] NEIDIG, RAY E. ACIDITY OF SILAGE MADE FROM VARIOUS CROPS. *In* Jour. Agr. Research, v. 14, no. 10, p. 395–409. 1918. Literature cited, p. 408–409.

[2] DUCLAUX, E. RECHERCHES SUR LES VINS. DEUXIÈME MÉMOIRE: SUR LES ACIDES VOLATILS DU VIN. *In* Ann. Chim. et Phys., s. 5, t. 2, p. 289–324. 1874.

——— TRAITÉ DE MICROBIOLOGIE. t. 3, p. 388. Paris, 1900.

[3] GILLESPIE, L. J., and WALTERS, E. H. THE POSSIBILITIES AND LIMITATIONS OF THE DUCLAUX METHOD FOR THE ESTIMATION OF VOLATILE ACIDS. *In* Jour. Amer. Chem. Soc., v. 39, no. 9, p. 2027–2055, 3 fig. 1917. Literature cited, p. 2055.

[4] DYER, D. C. A NEW METHOD OF STEAM DISTILLATION FOR THE DETERMINATION OF THE VOLATILE FATTY ACIDS, INCLUDING A SERIES OF COLORIMETRIC QUALITATIVE REACTIONS FOR THEIR IDENTIFICATION. *In* Jour. Biol. Chem., v. 28, no. 2, p. 445–473, 2 fig. 1917.

TABLE III.—*Acidity of 100 gm. sugar beet top silage containing moisture*

Sample No.	Moisture.	Dry material.	Acetic acid.	Propionic acid.	Butyric acid.	Valeric acid.	Total volatile acid.	Lactic acid.	Total acids.
	Per cent.	*Per cent.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	81.5	18.5	0.51	0	0.73	0	1.24	0.59	1.83
2	76.0	24.0	.42	0	.17	0	.59	.69	1.28
5	49.5	50.5	.17	0.15	.25	0	.57	Trace.	.57
6	69.5	30.5	.71	.05	1.13	0	1.89	1.71	3.60
7	70.0	30.0	0	0	.54	0.04	.58	Trace.	.58
8	70.0	30.0	.29	.10	0	0	.39	.41	.80
9	78.5	21.5	.31	.05	.44	0	.80	.26	1.06

TABLE IV.—*Acidity of 100 gm. sugar beet top silage on dry basis*

Sample No.	Acetic acid.	Propionic acid.	Butyric acid.	Valeric acid.	Total volatile acid.	Lactic acid.	Total acids.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	2.73	0	3.92	0	6.65	3.07	9.72
2	1.74	0	.70	0	2.44	2.79	5.23
5	.35	0.29	.50	0	1.14	Trace.	1.14
6	2.32	.17	3.70	0	6.19	5.61	11.80
7	0	0	1.79	0.12	1.91	Trace.	1.91
8	.96	.34	0	0	1.30	1.35	2.65
9	1.46	.25	2.04	0	3.75	1.21	4.96

In the samples of sugar beet top silage, sample 8 is the only one that contains the same acids that are found in corn silage. This silage was classed as a fair quality of silage by experts when it was sent to this station. The remaining samples of silage all contained some butyric acid. The quality of the silage ranged from fair to poor, depending chiefly upon the amount of dirt that was in the silage. While the amount of butyric acid present indicates in a degree the type of fermentation, it does not seem to prevent stock from eating the silage. Some samples contained butyric acid in quantities that made drying the material in an oven very unpleasant unless the process was carried on under a hood, and yet cattle ate the silage with relish. It is not known how much effect the abnormal fermentation has on the feeding value of silage, but no doubt some loss occurs. Such losses could be greatly reduced by carefully packing the beet tops when siloing and by covering the tops in such manner that all the air is excluded.

The mere presence of butyric acid in silage is not in itself harmful, but it is the fact that the presence of butyric acid indicates an abnormal fermentation, resulting in a partial decomposition of silage, which tends to lower its feeding value.

It is hardly to be expected that beet tops can be packed sufficiently to exclude all air, because of the nature of the tops, but possibly cutting the tops in a silage cutter would solve the problem. Experiments are planned for the coming year to determine the best methods of siloing sugar beet tops.

Lactic acid is present in very small amounts in many of the samples. It is possible that more lactic acid is present in the early stages of fermentation and that it is either changed into other acids or is decomposed. An additional investigation is needed to explain fully the reason for the small amounts of lactic acid in abnormal silage. The lactic acid present is the racemic mixture.

The fact that sample 8 contains the characteristic acids of normal silage indicates that sugar beet tops can be successfully siloed if proper precautions are taken to pack the tops well and exclude air. The samples of silage analyzed came from a pit silo ranging from 2½ feet to 8 feet in depth. Without question, depth of the pit silo is an important factor in the production of good silage. Where shallow silos are used, air gains access to the greater portion of the beet tops and a poor silage results, whereas, in the deeper silos there is less chance for the entire silage to be partially spoiled on account of access of air. It is important, then, to have a deep silo to eliminate dirt, and to pack thoroughly so as to exclude air. These precautions will insure a better average silage throughout the Northwest than is now found.

SUMMARY

(1) It is evident that the quality of sugar beet top silage put up by the average Idaho farmer is very poor.

(2) Large quantities of dirt are present, which could be eliminated in a large measure by careful handling of the product during siloing.

(3) To improve the quality of silage, pit silos should be deep and the silage should be packed thoroughly and covered sufficiently to exclude air. Excess dirt should be eliminated.

(4) More care should be taken by the average farmer in siloing sugar beet tops. While stock will eat silage that is very poor, there is a loss of food value in improperly made silage as well as danger of mortality.

NODULE BACTERIA OF LEGUMINOUS PLANTS

By F. Löhnis, *Soil Biologist, Bureau of Plant Industry, United States Department of Agriculture*, and Roy Hansen, *Professor of Soils, University of Saskatchewan, Saskatoon, Sask.*[1]

INTRODUCTION

Despite the fact that the nodule bacteria of the leguminous plants have been made the subject of numerous publications, it is not to be disputed that their true morphological and physiological character, as well as their correct systematic position, are by no means sufficiently known. This is especially clearly demonstrated by the fact that they are still proclaimed by several writers to be the representatives of a special genus Rhizobium, once established by A. B. Frank as the result of rather inadequate studies upon this subject. In the new classification of bacteria, adopted by the Society of American Bacteriologists, the nodule bacteria again are widely separated from closely related species, and the error concerning the so-called genus Rhizobium has been revived once more.

Comparative investigations upon the symbiotic and the nonsymbiotic nitrogen-fixing bacteria of the soil, published in 1905 by the senior author, have proved conclusively that the nodule bacteria are not representatives of a special genus Rhizobium, but that they are closely related to *Bacillus radiobacter* Beijerinck and further to *B. lactis viscosum* Adametz, *B. pneumoniae* Friedländer, and *B. aerogenes* Escherich. The last three organisms are immotile, while the first one is motile; but here again the very close relationship between the immotile *B. aerogenes* and the motile *B. coli* has to be kept in mind. In fact, there can be easily isolated from every soil numerous varieties of *B. radiobacter*, which lead gradually up to *B. coli*, acquiring the power of fermentation and that type of growth on solid substrates which is characteristic of the last-named species. It has been pointed out in detail that all species mentioned above differ only gradually, not principally, in their physiological and morphological qualities, and especially that those branched or otherwise changed cell forms which are frequent in the root nodules are equally common with all members of this group of capsule bacteria, if these are tested adequately.[2] The ability to fix the atmospheric nitrogen was shown to be common in this group of organisms.

[1] Most of the experiments discussed in this paper were made in the summer of 1919, at the University of Illinois, where at that time the junior author held the position of Associate in Soil Biology. The photographs accompanying the paper were made by Mr. F. L. Goll, of the Bureau of Plant Industry, United States Department of Agriculture.

[2] It is not superfluous to emphasize once more that persistence in calling these forms "bacteroids" is by no means to be recommended. They are true bacteria, not foreign bodies looking like bacteria, as Frank's pupil Brunchorst erroneously believed. To speak of a "bacteroid" growth of bacteria is no less absurd than it would be to speak of a "fungoid" growth of fungi.

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Bacillus radiobacter was found to be peritrichic, and the same paper also indicated (*12, p. 592, footnote*)[1] that in all probability *B. radicicola* has the same kind of flagellation. But no faultless preparates were obtained at that time.

In the same year, 1905, G. T. Moore wrote concerning the nodule bacteria (*14, p. 26*):

> There does not seem to be any necessity for creating a new group to include these organisms, as has been done by Frank, under the name of Rhizobium, for although there is a certain amount of polymorphism, it is no greater than frequently occurs in the bacteria.

With regard to the flagellation, however, Moore himself evidently made no special studies, and, accepting Beijerinck's statement that the "swarming bodies" (gonidia) of *Bacillus radicicola* are monotrichic as being valid for the bacteria too, he proposed to call the nodule bacteria *Pseudomonas radicicola*. Numerous authors have followed this suggestion, and experiments made by Harrison and Barlow (*8*) apparently confirmed the view that the flagellation of these organisms is indeed monotrichic.

However, these experiments are, in fact, not convincing, as has been emphasized especially by Kellerman (*9*). This author and also G. de Rossi (*16, 17*), Zipfel (*19*), and Prucha (*15*) secured results all of which demonstrated more or less clearly that the senior author's assumption was correct: *Bacillus radicicola* is peritrichic; it is no "Pseudomonas."

But this seemed again to be contradicted by certain results obtained by the junior author while working with the late T. J. Burrill (*6*). Numerous tests made with the bacteria isolated from cowpea, soybean, Japan clover, and other plants showed clearly and invariably monotrichic flagellation, and, therefore, the designation *Pseudomonas radicicola* was restored once more. Additional results, however, indicated that there are other features which differentiate the bacteria of the cowpea-soybean group from those living in the roots of clover, alfalfa, pea, and vetch. Especially the slime production and the speed of growth appeared to be different, and the organisms studied were arranged into two groups, "slow growers" and "fast growers" Both, however, were supposed to be merely varieties of *P. radicicola*.

This point remained to be investigated more thoroughly. In addition, another "fast grower" presented itself for detailed study, which quite regularly appeared on thickly sown plates of the "slow growing" groups, and which, indeed, has been mistaken by several investigators as the true nodule organism of cowpea, soybean, Japan clover, etc. Repeatedly such cultures were sent to and tested by the junior author. They were all unable to produce nodules.

The data given on the following pages make it evident that this "fast grower" is *Bacillus radiobacter*, which plays in this case, also, a very

[1] Reference is made by number (italic) to "Literature cited," p. 554–555.

interesting rôle. As this same species undoubtedly takes part in many processes occurring in soil and in water, it was thought useful to give another more detailed description of it, especially because, despite its ubiquity, *B. radiobacter* is much too little known. In addition to the rather short description given by Beijerinck, only the more complete one published by the senior author in 1905 exists thus far. On account of its great similarity to *B. radicicola*, *B. radiobacter* should be very well known to all bacteriologists working with the nodule bacteria in order to avoid mistakes which may otherwise not be discovered until only negative results are obtained in the inoculation tests.

Concerning the flagellation of the nodule bacteria three statements have been published more recently which also will have to be discussed presently. According to J. K. Wilson (*18*) the soybean bacteria are peritrichous; Barthel (*2*) declared lupine and alfalfa bacteria to be lophotrichous; Fred and Davenport (*7*) found the alfalfa organism peritrichous, but they found the lupine bacteria characterized by having one, rarely two, flagella.

EXPERIMENTAL RESULTS

The following strains of nodule bacteria were studied after having been tested with positive results in regard to their ability to produce nodules on the host plants from which they were isolated.

1. Cowpea.
2. Peanut.
3. Japan clover.
4. Beggar weed.
5. Soybean.
6. Red clover.
7. Sweet clover.
8. Vetch.
9. Strophostyles.

There were also included in our investigations two strains isolated from:

10. Black locust.
11. Lupine.

No positive inoculation test could be made on black locust. The lupine culture was kindly furnished by Dr. E. B. Fred, of the University of Wisconsin, who had tried it with positive results on this plant. Our tests were equally successful.

Two noninfectious "fast growing" cultures isolated from legume nodules and identified as *Bacillus radiobacter* were studied in comparison with six Radiobacter strains which originated from soil and which were kept in the senior author's collection since the years given in parentheses.

12. Fast grower from cowpea.
13. Fast grower from soybean.
14. *Bacillus radiobacter* from soil (1904).
15. Same (1907).
16. *Bacillus radiobacter* from soil (1908).
17. Same (1908).
18. Same (1908).
19. Same (1916).

No. 14 is the strain which in 1904 had been acknowledged by Prof. Beijerinck as being identical with his *Bacillus radiobacter* and which was used by the senior author for the original description published in 1905 (*12*).

TABLE I.—*Development of cowpea-soybean bacteria, Bacillus radicicola (from clover, vetch, etc.), and B. radiobacter*

Substrates.	Cowpea-soybean bacteria.
Mannite-nitrate agar slant.	MACROSCOPIC EXAMINATION.—Raised whitish to porcelain white, glossy layer. MICROSCOPIC EXAMINATION.—After 3 days slender rods, sometimes curved; after 7 to 10 days unstained, irregular sheaths, with 1 to 4, most frequently 2, darkly stained granules; after 2 to 3 weeks many small globules, ovals, and short rods outside of the unstained sheaths, also small globular regenerative bodies.
Beef agar slant.	MACROSCOPIC EXAMINATION.—Fairly good whitish growth. MICROSCOPIC EXAMINATION.—After 3 days weakly stained, irregular, thin, short rods; after 7 to 10 days irregular rods, producing gonidia and globular regenerative bodies, which may multiply as such; after 2 to 3 weeks very variable appearance, rather long slender rods, often branched, or club-shaped, globular regenerative bodies, also unstained, irregular sheaths with dark granules, and large globular gonidangia.
Beef gelatin stab.	MACROSCOPIC EXAMINATION.—Very small, gray, nonliquefying disk on the surface, hardly any growth in the stab. MICROSCOPIC EXAMINATION.—Thin rods, sometimes branched or swollen, producing gonidia and small globular regenerative bodies; in old cultures gonidia and regenerative bodies frequently predominating.
Beef broth.	MACROSCOPIC EXAMINATION.—Broth at first clear, with little sediment; later (after about 2 weeks) slightly turbid. MICROSCOPIC EXAMINATION.—After 3 days slender rods, sometimes curved; after 2 weeks granular rods producing gonidia, also budding and branching, small globular regenerative bodies, and symplasm; after 3 to 4 weeks very irregular forms, branching, swelling.
Milk.	MACROSCOPIC EXAMINATION.—During the first weeks no change visible, later slow digestion, no clear serum zone. MICROSCOPIC EXAMINATION.—Mostly small globules and ovals, few short, slender rods.
Potato.	MACROSCOPIC EXAMINATION.—Very meager, transparent, thin layer. MICROSCOPIC EXAMINATION.—After 7 days slender rods, sometimes branched, or with terminal swelling; after 4 weeks small globules and ovals, irregular rods (frequently branched), globular regenerative bodies, and symplasm with very variable new development.

TABLE I.—*Development of cowpea-soybean bacteria, Bacillus radicicola (from clover, vetch, etc.), and B. radiobacter*—Continued

Substrates.	*B. radicicola* (from clover, vetch, etc.).	*B. radiobacter.*
Mannite-nitrate agar slant.	MACROSCOPIC EXAMINATION.—Slimy, transparent growth, with or without whitish streaks. MICROSCOPIC EXAMINATION.—Small ovals and short rods, producing after 1 to 2 weeks gonidia and small globular regenerative bodies. Also unstained slime threads with dark granules and large globular, or oval gonidangia; irregular pale forms from symplasm.	MACROSCOPIC EXAMINATION.—Thick, slimy transparent layer, with whitish streaks. MICROSCOPIC EXAMINATIONS.—After 7 days small ovals and short rods, imbedded in slime; after 14 days some rods with thick unstained capsules forming symplasm; after 3 to 4 weeks normal cells, stars, and large globules and clubs from symplasm.
Beef agar slant.	MACROSCOPIC EXAMINATION.—Meager, flat, grayish growth. MICROSCOPIC EXAMINATION.—Mostly small ovals and short rods, the latter sometimes curved, budding and branching; later also large rods, and large globular, oval, or club-shaped gonidangia.	MACROSCOPIC EXAMINATION.—Flat, whitish slimy layer, thick sediment below. MICROSCOPIC EXAMINATION.—As on mannite-nitrate agar.
Beef gelatin stab.	MACROSCOPIC EXAMINATION.—Small, gray, nonliquefying disk on surface, very little growth in stab. MICROSCOPIC EXAMINATION.—Small ovals and short rods, gonidia, and small globular regenerative bodies.	MACROSCOPIC EXAMINATION.—Grayish, flat, round, nonliquefying surface growth, little growth in stab; after 2 to 4 weeks gelatine sometimes brown on top. MICROSCOPIC EXAMINATION.—Typical ovals and short rods, these sometimes curved or branched, also unstained slime threads with dark granules, later symplasm with stars.
Beef broth.	MACROSCOPIC EXAMINATION.—Broth either clear or very slightly turbid, little whitish sediment. MICROSCOPIC EXAMINATION.—Small ovals and short rods, budding and branching, occasionally threads; after 1 to 2 weeks many gonidia and small, globular regenerative bodies.	MACROSCOPIC EXAMINATION.—Broth turbid, white ring, whitish film, much whitish sediment. MICROSCOPIC EXAMINATION.—Small ovals and short rods, budding and branching; later gonidia, globular regenerative bodies, threads, and fine stars from symplasm.
Milk.	MACROSCOPIC EXAMINATION.—After 1 to 4 weeks clear serum zone on top, 2 to 5 mm. thick; milk below nearly unchanged, very fine flocculation. MICROSCOPIC EXAMINATION.—Small ovals and rods, later also granular threads and symplasm.	MACROSCOPIC EXAMINATION.—First slime ring and serum zone on top; later whole milk turning brown. MICROSCOPIC EXAMINATION.—After 7 days typical ovals and rods; later small and large cells from symplasm.
Potato.	MACROSCOPIC EXAMINATION.—Meager, transparent, slimy growth. MICROSCOPIC EXAMINATION.—Small slender rods, budding and branching, some ovals, globular regenerative bodies; later gonidia predominant.	MACROSCOPIC EXAMINATION.—First gray, later coli-brown slimy layer, potato turns frequently gray. MICROSCOPIC EXAMINATION.—First small ovals and short rods, budding and branching, later also large oval and globular gonidangia and symplasm with stars.

The results of our investigations leave no doubt that the earlier findings of the junior author were correct so far as the polar flagellation and the peculiar morphological and cultural features of the cowpea-soybean organisms are concerned. On the other hand, it could now be ascertained with equal certainty that the bacteria producing nodules on clover, alfalfa, vetch, and other plants originally cultivated in Europe are all peritrichic and exhibit all the characteristics of *Bacillus radicicola*, as described by Beijerinck and other authors.

Those findings which were obtained most frequently and which may be considered as being typical for the two groups of nodule bacteria are compiled in Table I, together with analogous data pertaining to *Bacillus radiobacter*. Photographs of the most characteristic details are reproduced on Plates 68 and 69.

When grown from the root nodule on Harrison and Barlow's ash agar, mannite agar, or similar agar of low nitrogen content, the two groups of nodule bacteria are best characterized and differentiated by the very slow growth of colonies in the cowpea-soybean group and the comparatively quick growth of those of *Bacillus radicicola* (*6, pl. 11, fig. 1-11*). Frequently, but not always, the development of *B. radiobacter* is still somewhat more rapid than that of *B. radicicola;* in the macroscopical as well as in the microscopical aspects, however, the colonies of these two species on such media are so very much alike that it is almost impossible to distinguish them with certainty. Both, when developing on the surface, are perfectly round, drop-like, soft, watery or slimy, glistening, transparent. Often a whitish center or whitish streaks become visible within the more transparent mass, especially if the surface colony is the outgrowth of an imbedded colony. Sometimes it appears as if this whitish center were regularly to be seen only with certain strains of Radicicola and Radiobacter. This is not the case, however. Its presence or absence is erratic and can not be used for differentiation. The imbedded colonies are always small, white, opaque, mostly lentiform, less frequently circular. Under the microscope the surface colonies present themselves as sharp-edged disks, pure white at the outside with yellowish-grayish granulation in the center. In a few cases a radiate structure becomes visible. The colonies of the cowpea-soybean group appear macroscopically, as well as microscopically like young colonies of the Radicicola type. The presence of Radiobacter colonies on the plate stimulates their growth markedly.

In cell morphology theré is again a more pronounced relationship between Radiobacter and Radicicola than between the nodule bacteria of the clover-vetch group on the one side and of the cowpea-soybean group on the other. This holds true with the regular rod forms as well as with the very pleomorphic, curved, swollen, branched, or otherwise changed types of growth characteristic of these groups. The photographs on Plate 68, D–I, represent the pictures we have seen most frequently, but they do not pretend to give a complete illustration of the wide pleomor-

phism of these organisms. Before their complete life history can be given much additional material will have to be collected, especially with regard to the form of gonidangia, regenerative bodies, and the various cells developing from the symplastic stage. At present we intend only to bring out as clearly as possible those points which are important for a correct differentiation and diagnosis. As far as one may judge from the microscopic appearance, it is the inclination of *Bacillus radiobacter* to form stars which is most characteristic (Pl. 68, L), and this was used, therefore, by Beijerinck for its denomination. With *B. radicicola* the frequent occurrence of the clear-cut, compact Y forms is the most conspicuous feature (Pl. 68, H); whereas the bacteria of the cowpea-soybean group present themselves in most cases, when stained with aqueous aniline dyes in the usual manner, as short or long, unstained sheaths with one or more darkly stained granules (Pl. 68, J). Of course Y forms, as well as unstained sheaths with darkly stained gonidia, can be observed not infrequently with the other organisms, too, and the star formation is by no means solely to be found with Radiobacter; but we feel sure that those pictures, as shown on Plate 68, G–L, will be found most valuable for diagnostical purposes.

The flagellation is the same with Radiobacter (Pl. 68, C) and Radicicola (Pl. 68, B), while the bacteria of the cowpea-soybean group are characterized by one coarse, fairly straight polar flagellum (Pl. 68, A). Just before fission one cilium may be seen at each end; as a rare exception a tuft of polar flagella was observed occasionally. Frequently a darkly stained body becomes visible within the rod just at that point where the flagellum springs forth, which may be considered to be a flagellated, not yet liberated, gonidium, such as can be seen occasionally with many other bacteria, especially with *Bacillus radicicola*, too. When liberated this becomes the monotrichic small "swarming body" described by Beijerinck in 1888 (*4*).

The growth on mannite-nitrate agar, as well as on beef agar slants, as described in Table I, is quite characteristic, and after the eyes have been sufficiently trained, one seldom makes a mistake in guessing the group to which a culture presented for inspection may belong. But it must be admitted that occasionally and temporarily a strain of the cowpea-soybean group can show the flat, transparent growth characteristic of Radicicola, whereas it is a very rare occurrence that a member of the last-named group simulates the former one. The growth of Radiobacter is always very typical, except when a very weak strain is encountered, which does not frequently occur within this group. Plate 69, A, demonstrates the characteristic differences noticeable on mannite-nitrate agar as clearly as they can be shown in a photographic reproduction.[1]

[1] As was the case with Azotobacter, for which the mannite-nitrate agar was first used (*13, p. 686*), so also the nodule bacteria and *Bacillus radiobacter* grew very readily on this substrate. Allen (*1, p. 33*) asserted recently that he could not get any growth of Azotobacter on a dextrose agar, which he erroneously called "Löhnis and Smith's medium." But not even the formula used by us has been quoted correctly by Allen, and it is, of course, quite obvious that on account of the alterations made by Allen his agar must indeed have been quite unsuitable.

Cultures on beef gelatine and in beef broth differentiate clearly Radiobacter and nodule bacteria, while, as stated in Table I, the two groups of nodule organisms grow very much alike on these substrates. Microscopic tests, however, made from gelatine and broth furnish, in most cases, especially characteristic pictures, provided that the growth has not been altogether too poor to get a satisfactory preparate.

The growth in milk and on potato, as described in Table I and illustrated on Plate 69, is very characteristic and can be used to great advantage for diagnosis. It is not to be denied that with old stock cultures atypical results may sometimes be obtained in this direction also. Especially cultures rich in or entirely made up of the globular regenerative bodies, which are produced by these as well as by all other bacteria, furnish whitish, yellowish, or only slightly brownish growth on potato in the case of *Bacillus radiobacter* and *B. radicicola.* But we have never seen such atypical growth with new isolations. Here the coli-brown color of the potato cultures separates Radiobacter sharply from the nodule bacteria, and these in turn are equally sharply to be distinguished by the behavior of their milk cultures. It is true that sometimes milk cultures of the *B. radicocola* group also leave the milk unchanged, but the microscopic test of such abnormal cases probably will always show, as it did in the cases studied by us, that the abnormality was simply caused by the fact that the bacteria which were inoculated did not multiply at all. Furthermore, no alteration may be seen if milk is used which has been kept for a long time and has become concentrated by evaporation of part of its water.

To determine on a larger scale whether this different behavior of the two groups of nodule bacteria, when grown in milk, can be correctly accepted as of real diagnostic value, all cultures of nodule bacteria at our disposal were tested simultaneously with the following results:

MILK WAS CHANGED AS TYPICAL FOR BACILLUS RADICICOLA BY THE FOLLOWING CULTURES:	MILK WAS LEFT UNCHANGED BY THE FOLLOWING CULTURES:
5 from red clover.	10 from cowpea.
4 from sweet clover.	8 from soybean.
6 from navy bean.	5 from peanut.
1 from vetch.	4 from Japan clover.
2 from lupine.	2 from beggar weed.
3 from black locust.	2 from *Cassia chamaecrista.*
3 from Amorpha.	
2 from Strophostyles.	

If kept for longer than four weeks milk cultures of the cowpea-soybean organisms usually become more or less transparent on account of partial decomposition of the casein; but they never show the perfectly clear zone characteristic of the other group.

The bacteria were also tested on other media besides the standard substrates, of which sterilized soil, moistened with 0.5 per cent mannite

solution, mannite-nitrate solution as used for studying the life cycle of Azotobacter, tap water plus 0.5 per cent beef broth, and 2 per cent salt agar furnished the most satisfactory results, especially with regard to a more complete knowledge of the cell morphology of the organisms. For diagnostic purposes, however, these substrates are of minor importance, as they do not bring out anything which is not already to be seen on the standard media. Nevertheless, it should be pointed out that cultures of the nodule bacteria in soil are to be recommended for two reasons. First, they are useful in keeping the organisms in a normal state of virility for a long time, and, in the second place, they demonstrate very clearly, when studied microscopically, that it is erroneous to believe—though numerous authors have promoted such hypotheses—that the nodule bacteria behave very differently in soil and could, therefore, not be isolated in their typical form from their natural habitat. Our results are in complete agreement with those recently obtained by Barthel (*3*) concerning the growth of bacteria in sterilized soil.

Tap water containing 0.5 per cent beef broth gave also very good development and proved repeatedly helpful in reviving old, weakened strains which refused to grow on solid substrates.

DISCUSSION

Our experimental results leave no doubt that the nodule bacteria of the leguminous plants are to be divided at least into two distinct groups, differing morphologically as well as culturally. It is equally beyond dispute that these differences are so marked and constant that one might be inclined to establish the nodule organism of the cowpea-soybean group as a new species. On account of its behavior in the inoculation test O. Kirchner has considered the soybean organism a distinct species, which he named in 1895 *Rhizobacterium japonicum*(*10*). According to the rules of priority, this species name would have to be given preference, despite the fact that different behavior in the inoculation test generally can not be accepted as a distinguishing mark of such quality as to validate the creation of a new species. The generic name Rhizobacterium, used by Kirchner, is, of course, equally as untenable as the generic name Rhizobium. According to the two tmost requently used modes of classifying the bacteria, one might name the cephalotrichic non-sporulating rod of the cowpea-soybean group *Pseudomonas japonica* or *Bacterium japonicum*, while the name *Bacterium* or *Bacillus radicicola* would have to be retained for the peritrichic organisms to be found with clover, alfalfa, sweet clover, vetch, pea, etc.

However, we do not advocate such a procedure. We are firmly of the opinion that much more must be known of the complete life history of a bacterium than is obtainable along the standardized lines of customary bacteriological research, before one can safely proceed to establish a genuine species on a truly scientific basis. Undoubtedly many if

not most of the commonly used so-called species names of bacteria are no species names at all, but merely denominations more or less correctly applied to organisms about whose complete life history and, accordingly, about whose true systematic value and position comparatively little is known at present.

It is by no means impossible that future systematic investigations may demonstrate the peritrichic and the cephalotrichic nodule bacteria to be relatively constant types of growth of one species. There are a few reports in the literature indicating that occasionally cross inoculations have been obtained, which might support this hypothesis. While O. Kirchner never found nodules on soybeans grown in Germany and therefore thought his *Rhizobacterium japonicum* to be the active agent in the Far East, F. Cohn said in a note appended to Kirchner's report that soybeans grown for the first time in his experimental garden in Breslau did produce nodules, though these were not of the normal type and contained only a few rodlike bacteria. Kellerman reported upon a case where a culture originally isolated from alfalfa was found to be infective on alfalfa and lupine as well as on soja when tested by Leonard after six years' cultivation on artificial substrates. It may be mentioned also in this respect that cross inoculations between navy bean and cowpea seem to be equally possible, under circumstances, however, which need further elucidation.

But just as negative results in cross inoculation experiments can not be accepted as sufficient basis for establishing different species, so also such rather exceptional positive results can not be used as valid support of the hypothesis that monotrichic and peritrichic nodule bacteria are only two types of growth of the same species. First of all, it would have to be ascertained whether in such cases the peritrichic organism has really changed into the monotrichic one, or vice versa. The possibility remains, of course, that occasionally the one type of organisms may invade a host plant whose nodules are normally caused by the other type of bacteria.

Changes in flagellation from peritrichic to cephalotrichic have been observed, according to Lehmann and Neumann (*11, p. 256, 357, 371*), with *Bacillus coli* and *B. alcaligenes*. Both species are related to *B. radiobacter* and *B. radicicola*, and under this aspect an analogous change should not be rejected prematurely as *a priori* improbable.

At the end of the introduction three statements have been quoted from the more recent literature which one might be inclined to accept as confirmative evidence in this direction. However, on account of the following reasons we do not feel justified in advocating such an interpretation.

J. K. Wilson says that in his preparations of soybean organism—

> The flagella were peritrichous, the highest number found being four.

As no photomicrographs had been made, Dr. Wilson was kind enough to furnish, on request of the senior author, one of his slides for examina-

tion. The flagella visible therein were all very weakly stained, so that no definite conclusion could be drawn. A culture, for which we are also indebted to Dr. Wilson, behaved in our hands like all those tested before; practically all cells were distinctly monotrichous. A comparison of Plate 68, A, with the pictures published on Plates IV and V of Bulletin 202, Illinois Agricultural Experiment Station (6), leaves no doubt about this point.

In Barthel's paper (*2, p. 16*) two drawings and one photomicrograph are to be found which clearly illustrate the following statement:

Bei den Lupinenbakterien sind die Geisseln ziemlich lang, wellig geformt und an einem Pole befestigt. Ihre Anzahl variiert von 1 bis 6. Ihre Placierung ist recht eigentümlich. Sie sitzen nämlich öfters nicht gerade an der Spitze des Zellleibes, sondern sozusagen an den "Ecken" und oft etwas von dem Hinterende entfernt. Oft findet man auch eine Geissel an der einen "Hinterecke" und mehrere andere zusammen an der anderen. . .

Bei den Luzernebakterien waren die Geisseln meist weniger und kürzer, am häufigsten 1 oder, seltener 3 oder 4, aber auch hier deutlich lophotrich. . .

Fred and Davenport (7), on the other hand, saw only one or two cilia with the lupine bacteria, while several strains of alfalfa organisms left no doubt as to their peritrichic flagellation.

We believe that these conflicting views are in fact not so irreconcilable as they seem to be. If well-made slides are examined carefully, some cells will always be discovered which clearly show that on account of the primary swelling and the following shrinking of their capsules, the flagella are often more or less dislocated. Some of the cells shown in Plate 68, A–C, exhibit this phase as clearly as it is possible in such reproductions. The flagella of the monotrichous bacteria of the cowpea-soybean group are to be seen in an exactly polar position only when the cells themselves are lying lengthwise within the "drift," as indicated by the floating flagella. In all other cases dislocations may take place, removing the cilia to the corners or even to the side of the cells, where they should not be viewed, however, as remnants of a peritrichic flagellation.

On the other hand, analogous disturbances may cause the occurrence of apparently cephalotrichic bacteria among the peritrichic cells of *Bacillus radicicola* and *B. radiobacter*. That there exists no truly polar flagellation in these cases, however, is evidenced by the fact that the cilia composing such an apparently polar tuft do not protrude exactly from the same spot, as they do, for example, in the cell with several polar flagella shown in Plate 68, A. They are always more or less separated and are only accidentally drawn together in the course of the shrinking of the capsule. A thorough examination of well-made preparations leaves no doubt that the original position of the flagella is peritrichic.

SUMMARY

(1) The nodule bacteria of the leguminous plants are to be divided into two groups, differing morphologically as well as physiologically.

(2) The first group shows all features characteristic of *Bacillus radicicola* Beijerinck. It is peritrichic, grows relatively fast on agar plates, and changes the milk in a very characteristic manner. It produces nodules on the roots of the following plants: clover, sweet clover, alfalfa, vetch, pea, navy bean, lupine, black locust, Amorpha, and Strophostyles.

(3) The second group is characterized by monotrichic flagellation, comparatively very slow growth on agar plates, and inability to cause a marked change in milk. It has been isolated from cowpea, soybean, peanut, beggarweed, Acacia, Genista, and Cassia.

(4) According to the customary manner of classifying bacteria, this second group of nodule bacteria would have to be considered to be a new species, and according to the rules of priority, it would have to be named *Pseudomonas japonica* or *Bacterium japonicum* (Kirchner). But we do not advocate such a procedure, because only a complete study of the life history of these two groups of organisms would make it possible to say definitely whether they are, indeed, two distinct species or merely different types of growth of the same organism.

(5) *Bacillus radicicola* is closely related to *B. radiobacter*. The generic name Rhizobium is to be rejected. The correct systematic position of both species is in the neighborhood of *B. aerogenes* and *B. coli*.

(6) *Bacillus radiobacter* seems to be regularly present in the root nodules of leguminous plants, stimulating development and activity of the nodule bacteria. On account of its similarity to *B. radicicola*, it has been repeatedly mistaken for the nodule-producing organism in the cowpea-soybean group, whose bacteria it outranks very considerably in the development on the plates made from the nodules. By its brown growth on potato, *B. radiobacter* can be easily differentiated from *B. radicicola*.

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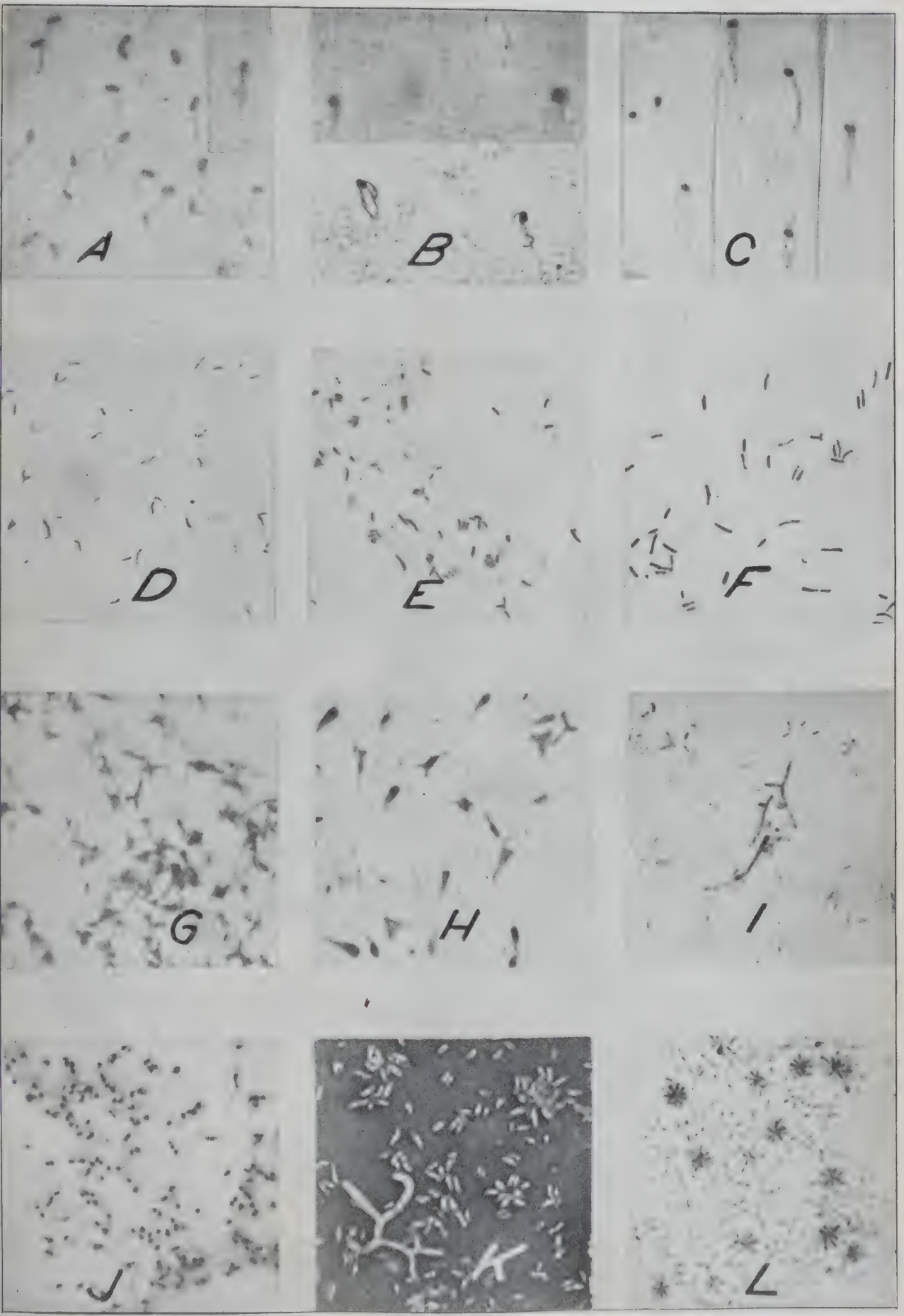
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PLATE 68

A.—Soybean bacteria, J. K. Wilson's strain, 4 days old.
B.—Vetch bacteria, 3 days old.
C.—Bacillus radiobacter, 2 days old.
D.—Soybean bacteria, beef agar, 4 days old.
E.—Red clover bacteria, beef agar, 4 days old.
F.—*Bacillus radiobacter*, beef agar, 4 days old.
G.—Cowpea bacteria, potato, 6 days old.
H.—Red clover bacteria, potato, 14 days old.
I.—*B. radiobacter*, milk, 7 days old.
J.—Cowpea bacteria, mannite-nitrate agar, 8 days old.
K.—Vetch bacteria, mannite-nitrate agar, 8 days old.
L.—*B. radiobacter*, mannite-nitrate solution, 17 days old.

A–C Loeffler's stain; D–L aqueous fuchsin. ×1,000.


A
B
C
D
E
F
G
H
I
J
K
L

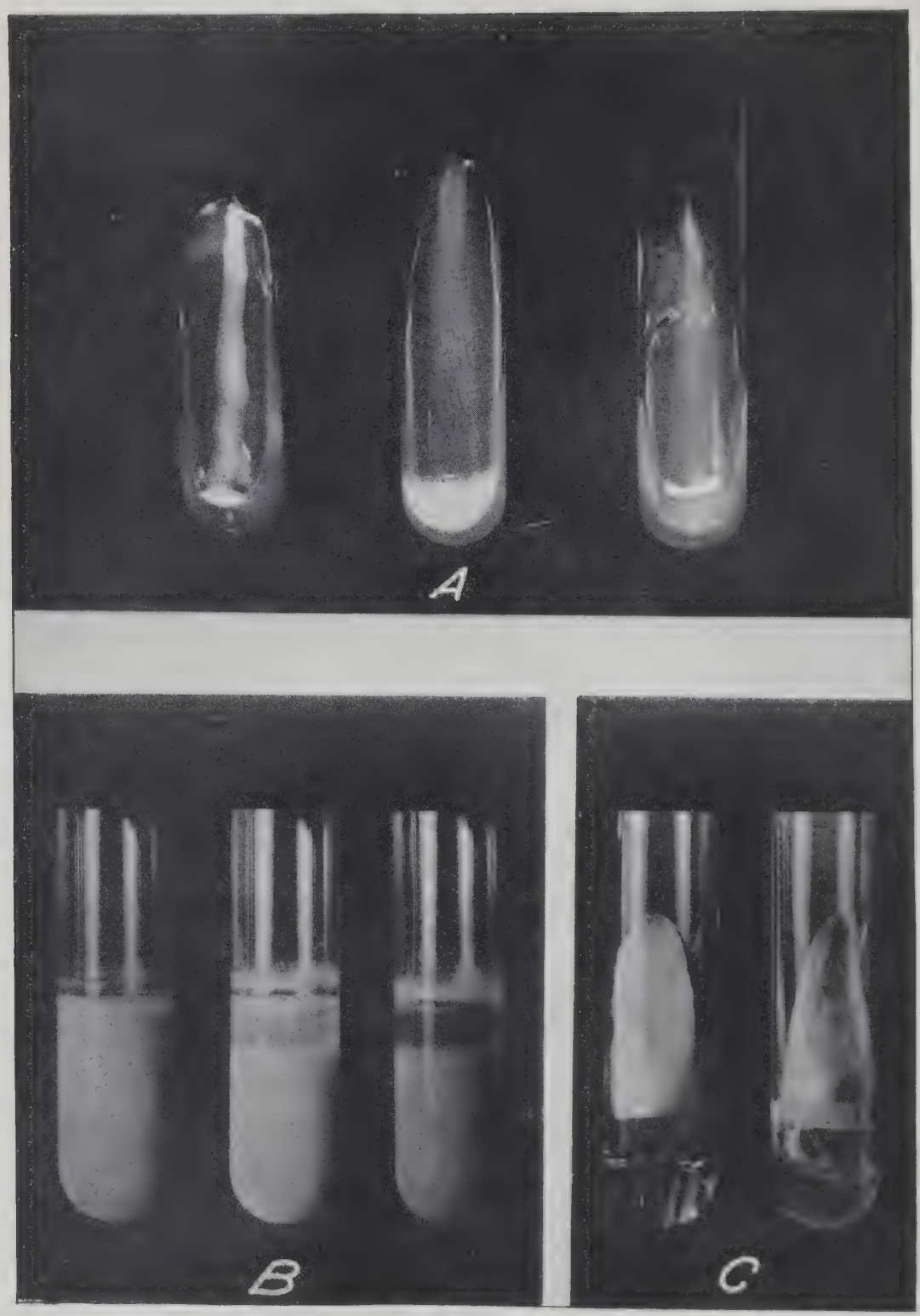

A
B
C

PLATE 69

A.—Mannite-nitrate agar slants, 8 days old, from left to right: soybean bacteria, vetch bacteria, and *Bacillus radiobacter*.

B.—Growth in milk, 4 weeks old from left to right: soybean bacteria, vetch bacteria, and *B. radiobacter*.

C.—Growth on potato, 2 weeks old: vetch bacteria (left) and *B. radiobacter* (right).

CORRELATION AND CAUSATION

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PART I. METHOD OF PATH COEFFICIENTS

INTRODUCTION

The ideal method of science is the study of the direct influence of one condition on another in experiments in which all other possible causes of variation are eliminated. Unfortunately, causes of variation often seem to be beyond control. In the biological sciences, especially, one often has to deal with a group of characteristics or conditions which are correlated because of a complex of interacting, uncontrollable, and often obscure causes. The degree of correlation between two variables can be calculated by well-known methods, but when it is found it gives merely the resultant of all connecting paths of influence.

The present paper is an attempt to present a method of measuring the direct influence along each separate path in such a system and thus of finding the degree to which variation of a given effect is determined by each particular cause. The method depends on the combination of knowledge of the degrees of correlation among the variables in a system with such knowledge as may be possessed of the causal relations. In cases in which the causal relations are uncertain the method can be used to find the logical consequences of any particular hypothesis in regard to them.

CORRELATION

Relations between variables which can be measured quantitatively are usually expressed in terms of Galton's (*4*)[1] coefficient of correlation, $r_{XY}=\frac{\Sigma X'Y'}{n\sigma_X\sigma_Y}$ (the ratio of the average product of deviations of X and Y to the product of their standard deviations), or of Pearson's (*7*) correlation ratio, $\eta_{X\cdot Y}=\frac{\sigma_{\left(M_X^Y\right)}}{\sigma_X}$ (the ratio of the standard deviation of the mean values of X for each value of Y to the total standard deviation of X), the standard deviation being the square root of the mean square deviation.

Use of the coefficient of correlation (r) assumes that there is a linear relation between the two variables—that is, that a given change in one variable always involves a certain constant change in the corresponding average value of the other. The value of the coefficient can never exceed

[1] Reference is made by number (italic) to "Literature cited," p. 585.

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+1 or −1. For many purposes it is enough to look on it as giving an arbitrary scale between +1 for perfect positive correlation, 0 for no correlation, and −1 for perfect negative correlation.

The correlation ratio (η) equals the coefficient of correlation if the relation between the variables is exactly linear. It does not, however, depend on the assumption of such a relation, and it is always larger than r when the relations are not exactly linear. It can only take values between 0 and +1, and it can be looked upon as giving an arbitrary scale between 0 for no correlation and 1 for perfect correlation.

The numerical value of the coefficient of correlation (r) takes on added significance in connection with the idea of regression. It gives the average deviation of either variable from its mean value corresponding to a given deviation of the other variable, provided that the standard deviation is the unit of measurement in both cases. The regression in terms of the actual units can, of course, be obtained by multiplying by the ratio of the standard deviations. Thus, for the deviation of X corresponding to a unit deviation of Y, we have $reg_{X \cdot Y} = r_{XY}\frac{\sigma_X}{\sigma_Y}$. This formula may be deduced from the theory of least squares as the best linear expression for X in terms of Y. The formula for what Galton later called the coefficient of correlation was, in fact, first presented in this connection by Bravais (*1*) in 1846. Any such interpretation is of course impossible with the correlation ratio.

The numerical values of both coefficients, however, have significance in another way. Their squares (η^2, or r^2 if regression is linear) measure the portion of the variability of one of the variables which is determined by the other and which disappears in data in which the second is constant. Thus if ${}_Y\sigma^2_X$ is the mean square deviation of X for constant Y, Pearson has shown that:

$$ {}_Y\sigma^2_X = \sigma^2_X(1-\eta^2_{X \cdot Y}) $$

or ${}_Y\sigma^2_X = \sigma^2_X(1-r^2_{XY})$ if regression is linear.

It often happens that it is desirable to consider simultaneously the relations in a system of more than two variables. For such cases, involving only linear relations between the various pairs of variables, Pearson (*6*) has devised the coefficient of multiple correlation.

$$ R_{X(ABC\cdots N)} = \sqrt{1-\frac{\Delta}{\Delta_{XX}}} $$

in which

$$ \Delta = \begin{vmatrix} 1 & r_{XA} & r_{XB} & \cdots & r_{XN} \\ r_{AX} & 1 & r_{AB} & \cdots & r_{AN} \\ r_{BX} & r_{BA} & 1 & \cdots & r_{BN} \\ \cdot & \cdot & \cdot & \cdots & \cdot \\ \cdot & \cdot & \cdot & \cdots & \cdot \\ r_{NX} & r_{NA} & r_{NB} & \cdots & 1 \end{vmatrix} $$

and Δ_{XX} is the minor made by deleting row X and column X. $R^2_{X(ABC\cdots N)}$ measures the degree of determination of X by the whole set of other factors, and $1-R^2_{X(ABC\cdots N)}=\frac{\Delta}{\Delta_{XX}}$ is the maximum possible squared correlation between X and a factor independent of those considered. This formula for multiple correlation leads to one for multiple regression. Letting X', A', B', etc., be the deviations of variables X, A, B, etc., from their mean values, Pearson has shown that the most probable value of X' for known values of the other variables is given by the formula

$$\frac{X'}{\sigma_X}=\frac{\Delta_{XA}}{\Delta_{XX}}\frac{A'}{\sigma_A}+\frac{\Delta_{XB}}{\Delta_{XX}}\frac{B'}{\sigma_B}\quad\cdots\quad\frac{\Delta_{XN}}{\Delta_{XX}}\frac{N'}{\sigma_N}$$

$$\sigma_X{}'=_{N\cdots BA}\sigma_X=\sigma_X\sqrt{\frac{\Delta}{\Delta_{XX}}}.$$

Analogous but more complex formulae have recently been published by Isserlis (*5*) for the multiple correlation ratio for use in cases in which the regressions are not necessarily linear.

CAUSATION

In all the preceding results no account is taken of the nature of the relationship between the variables. The calculations thus neglect a very important part of the knowledge which we often possess. There are usually *a priori* or experimental grounds for believing that certain factors are direct causes of variation in others or that other pairs are related as effects of a common cause. In many cases, again, there is an obvious mathematical relationship between variables, as between a sum and its components or between a product and its factors. A correlation between the length and volume of a body is an example of this kind. Just because it involves no assumptions in regard to the nature of the relationship, a coefficient of correlation may be looked upon as a fact pertaining to the description of a particular population only to be questioned on the grounds of inaccuracy in computation. But it would often be desirable to use a method of analysis by which the knowledge that we have in regard to causal relations may be combined with the knowledge of the degree of relationship furnished by the coefficients of correlation.

The problem can best be presented by using a concrete example. In a population of guinea pigs it will be found that the birth weights, early gains, sizes of litters, and gestation periods are all more or less closely correlated with each other. The influence of heredity, environmental conditions, health of dam, etc., are also easily shown. In a rough way, at least, it is easy to see why these variables are correlated with each other. These relations can be represented conveniently in a diagram like that in figure 1, in which the paths of influence are shown by arrows.

The variety and complexity of the relations which may be back of a correlation are well illustrated in this case. Thus, the weight at weaning (33 days of age) should be correlated with the birth weight and with the gain between birth and weaning simply because it is their sum. The relations of birth weight with gestation period and the prenatal rate of growth are also essentially mathematical rather than causal. Birth weight is necessarily fully determined by the character of the prenatal growth curve and the time at which this is interrupted by birth.

In the relation between gestation period and size of litter we come to a case in which there is no necessary mathematical relationship. We naturally attempt to account for the high negative correlation by the hypothesis that a large number in a litter in some way causes early

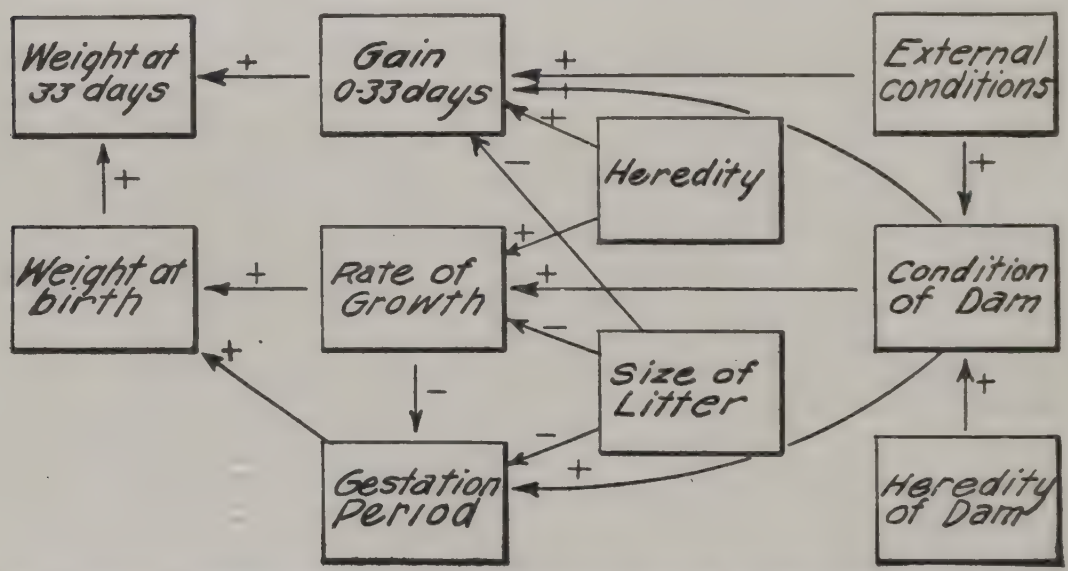


FIG. 1.—Diagram illustrating the interrelations among the factors which determine the weight of guinea pigs at birth and at weaning (33 days).

parturition. Similarly, a large number in a litter might be expected to be a cause of slow growth in the foetuses.

Birth weight and gain after birth are highly correlated. Here neither variable can be spoken of as the cause of variation in the other, and the relation is not mathematical. They are evidently influenced by common causes, among which heredity, size of litter, and conditions which affect the health of the dam up to the time of birth at once come to mind.

Most of the variables are connected with each other through more than one path. Thus, weight at birth is correlated with weight at weaning both as a component of a sum and as the effect of common causes.

There may be a conflict of the paths. Thus, a large number in a litter has a fairly direct tendency to shorten the gestation period, but this is probably balanced in part by its tendency to reduce the rate of growth of the foetuses, slow growth permitting a longer gestation period. Large litters tend to reduce gestation period and rate of growth before and after birth. But large litters are themselves most apt to come when

external conditions are favorable, which also favors long gestation periods and vigorous growth.

The coefficient of correlation is a resultant of all paths connecting the two variables. It would be valuable in many cases to be able to determine the relative importance of each particular path. The usual method in such cases is to calculate the partial correlation between two variables for a third constant, using Pearson's well-known formula

$$_{C}r_{AB} = \frac{r_{AB} - r_{AC}r_{BC}}{\sqrt{(1 - r^2_{AC})(1 - r^2_{BC})}}$$

for correlation between A and B for constant C. Such partial correlations, however, must be interpreted with caution. It is true that by making constant a connecting link between two variables, whether it is a common cause or the cause of one and effect of the other, we eliminate the path in question. This elimination of connecting paths in which the constant factor is a link is not, however, the only way in which correlation is affected. If an effect of a number of causes is made constant, spurious negative correlations appear among the causes and their other effects. Thus, if weight at 33 days is made constant, the correlation between birth weight and gain necessarily becomes -1. We are simply picking out a population in which any deficiencies in birth weight happen to be exactly balanced by excess in gain after birth. This is an extreme case, but where the relations of cause and effect are at all complex it is evident that the correlation between two variables may be changed in more than one way by making a third variable constant, making the interpretation doubtful.

Where there is a network of causes and effects, the interrelations could be grasped best if a coefficient could be assigned to each path in the diagram designed to measure the direct influence along it. The following is an attempt to provide such a coefficient, which may be called a path coefficient.

DEFINITIONS

We will start with the assumption that the direct influence along a given path can be measured by the standard deviation remaining in the effect after all other possible paths of influence are eliminated, while variation of the causes back of the given path is kept as great as ever, regardless of their relations to the other variables which have been made constant. Let X be the dependent variable or effect and A the independent variable or cause. The expression $\sigma_{X \cdot A}$ will be used for the standard deviation of X, which is found under the foregoing conditions, and may be read as the standard deviation of X due to A. In a system in which variation of X is completely determined by A, B, and C we have $\sigma_{X \cdot A} = {}_{A}r_{CB}\sigma_{X}$ representing the constant factors, B and C, and also the variation of A itself (σ_{A}) by subscripts to the left. The path

coefficient for the path from A to X will be defined as the ratio of the standard deviation of X due to A to the total standard deviation of X.

$$p_{X\cdot A}=\frac{\sigma_{X\cdot A}}{\sigma_X}.$$

Just as the regression of X on A is expressed by $r_{XY}\frac{\sigma_X}{\sigma_A}$ the deviation of X directly caused by a unit deviation of A is given by the formula

$$p_{X\cdot A}\frac{\sigma_X}{\sigma_A}=\frac{\sigma_{X\cdot A}}{\sigma_A}.$$

Another coefficient which it will be convenient to use, the coefficient of determination of X by A, $d_{X\cdot A}$, measures the fraction of complete determination for which factor A is directly responsible in the given system of factors. This definition implies that the sum of such coefficients must equal unity if all causes are accounted for.

SYSTEMS OF INDEPENDENT CAUSES

The degree of determination of one variable by another is most easily found where the variables are connected by a mathematical relationship. The simplest mathematical relationship is that between a sum and its components. For the standard deviation of a sum the following relation is well known:

$$\sigma^2_{A+B}=\frac{\Sigma(A'+B')^2}{n}=\sigma^2_A+\sigma^2_B+2\sigma_A\sigma_B r_{AB}.$$

If A and B are independent of each other, $r_{AB}=0$, and we have

$$\sigma^2_{A+B}=\sigma^2_A+\sigma^2_B.$$

The degree to which variation of the sum is determined by that of each component is obvious.

$$d_{X\cdot A}=\frac{\sigma^2_A}{\sigma^2_X} \text{ and } d_{X\cdot B}=\frac{\sigma^2_B}{\sigma^2_X}, \text{ where } X=A+B,$$

giving $d_{X\cdot A}+d_{X\cdot B}=1$, as required by definition.

For the standard deviation of X due to A we have in this case, $\sigma_{X\cdot A}=\sigma_A$. Thus, $p_{X\cdot A}=\frac{\sigma_{X\cdot A}}{\sigma_X}=\frac{\sigma_A}{\sigma_X}$ by definition.

Again, $r_{XA}=\frac{\Sigma(A'+B')A'}{n\sigma_X\sigma_A}=\frac{\Sigma A'^2}{n\sigma_X\sigma_A}=\frac{\sigma_A}{\sigma_X}$.

Summing up, $p_{X\cdot A}=\sqrt{d_{X\cdot A}}=r_{XA}$.

It can easily be shown that the same formulae hold in case we are dealing with the sum of multiples of a number of independent factors instead of with their own sum.

We can pass at once from this case to cases in which variation of X is caused in the physical or physiological sense by variation in several causes

provided that these causes are independent of each other, have linear relations to the dependent variable X, and that the deviations which they determine are additive. They are independent of each other if there is no correlation between their variations. A cause has a linear relation to the effect and is combined additively with the other factors if a given amount of change in it always determines the same change in the effect, regardless of its own absolute value or that of the other causes. The conclusion is that, under these conditions, the path coefficient equals the coefficient of correlation between cause and effect, and the degree of determination equals the square of either of the preceding coefficients.

CHAINS OF CAUSES

If we know the extent to which a variable X is determined by a certain cause M, which is independent of other causes, combines with them additively, and acts on X in a linear manner, and if we know the extent to which M is determined by a more remote cause A, the degree of determination of X by A must be the product of the component degrees of determination.

Let $X = M + N$, and $M = A + B$

$$d_{X \cdot M} = \frac{\sigma^2_M}{\sigma^2_X},\ d_{M \cdot A} = \frac{\sigma^2_A}{\sigma^2_M},\ \text{and}\ d_{X \cdot A} = \frac{\sigma^2_A}{\sigma^2_X}.$$

Thus $d_{X \cdot A} = d_{X \cdot M} d_{M \cdot A}$

and $p_{X \cdot A} = p_{X \cdot M} p_{M \cdot A}$.

NONADDITIVE FACTORS

In cases in which a factor does not act additively with the other factors in determining the variations in the dependent variable, its influence on the latter can not be completely expressed apart from the other factors, at least in terms of the ordinary measures of variability. This can be made clearer by an illustration. Multiplying factors are among the most important of those which do not combine by addition.

Let $X = AB$ and assume that $r_{AB} = o$

$$\sigma^2_X = M^2_B \sigma^2_A + M^2_A \sigma^2_B + \frac{\Sigma A'^2 B'^2}{n}$$

where A' and B' are deviations of A and B from their mean values M_A and M_B. Putting B constant, we have $\sigma^2_{X \cdot A} = M^2_B \sigma^2_A$; and similarly putting A constant, we have $\sigma^2_{X \cdot B} = M^2_A \sigma^2_B$. There remains a portion of σ^2_X which is due to A and B jointly and which can not be separated into parts due to each alone. If we write $d_{X \cdot A} = \frac{M^2_B \sigma^2_A}{\sigma^2_X}$ as the degree of determination of X by variation of A alone, and $d_{X \cdot B} = \frac{M^2_A \sigma^2_B}{\sigma^2_X}$ as the corresponding degree of determination of X by variation of B alone, we must recognize an additional term $d_{X \cdot \overline{AB}} = \frac{\Sigma A'^2 B'^2}{n \sigma^2_X}$, in order that the sum of the

coefficients of determination may equal unity. Regression is linear and $r^2_{XA}=\eta^2_{X\cdot A}=\frac{M^2_B\sigma^2_A}{\sigma^2_X}$. Thus $d_{X\cdot A}=r^2_{XA}$ as in the case of independent additive factors. The term $\frac{\Sigma A'^2B'^2}{n\sigma^2_X}$ is small unless the amounts of variation in A and B are large in comparison with the mean values. In many cases it is safe to deal with path coefficients and degrees of determination in the case of multiplying factors just as in the case of additive factors.

As a concrete illustration of these points take two independent variables, for each of which the values 1, 2, and 3 occur in the frequencies 1, 2, and 1, respectively. Below is the correlation table between one of these factors and their product.

Product (X).

Factor (A).	1	2	3	4	5	6	7	8	9	
1.....	1	2	1	....	....	....	....	....	....	4
2.....	....	2	....	4	....	2	....	....	....	8
3.....	....	....	1	....	....	2	....	....	1	4
	1	4	2	4	0	4	0	0	1	16

$M_A=2 \quad \sigma_A=\sqrt{1/2} \quad r_{AX}=\sqrt{8/17} \quad d_{X\cdot A}=8/17$

$M_X=4 \quad \sigma_X=\sqrt{17/4} \quad \frac{\Sigma A'^2B'^2}{n\sigma^2_X}=1/17 \quad d_{X\cdot B}=8/17$

$d_{X\cdot\overline{AB}}=\frac{1/17}{1}$.

In this case the amounts of variation in the factors are relatively large compared with their mean values, making the distribution surface markedly heteroscedastic, yet the degree of determination by either factor comes out only slightly less than one-half.

NONLINEAR RELATIONS

Pearson's definition of the correlation ratio, $\eta_{X\cdot A}=\frac{\sigma({}_AM_X)}{\sigma_X}$, has already been given. The variations of the mean value of X for different values of A are the variations which can be attributed to the direct influence of A, assuming that A is cause, X effect, and that other causes are combined with A additively. Thus $\sigma_{X\cdot A}=\sigma({}_AM_X)$ and we have at once $p_{X\cdot A}=\eta_{X\cdot A}$.

Again, as the total variation of X is composed of the variation of its mean values for different values of A, plus the variation about these mean values, we have $\sigma^2_X=\sigma^2({}_AM_X)+{}_A\sigma^2_X$, giving ${}_A\sigma^2_X=\sigma^2_X\,(1-\eta^2_{X\cdot A})$, as already noted.

Thus $\eta^2_{X\cdot A}$ measures the portion of σ^2_X lost by making A constant, so that as before $d_{X\cdot A}=\eta^2_{X\cdot A}=p^2_{X\cdot A}$.

Unfortunately we can not deal with chains of factors which involve nonlinear relations by mere multiplication of the path coefficients of the component links. In the present paper, unless otherwise stated, it will be assumed that all correlations are essentially linear.

EFFECTS OF COMMON CAUSES

Suppose that two variables, X and Y, are affected by a number of causes in common, (B, C, D). Let A represent causes affecting X alone and E causes affecting Y alone (fig. 2).

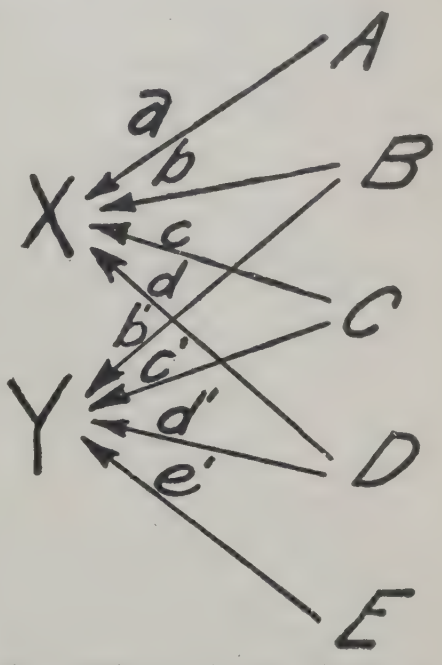


FIG. 2.—Diagram showing relations between two variables, X and Y, whose values are determined in part by common causes, B, C, and D, which are independent of each other.

Let

$p_{X \cdot A} = a$	$p_{Y \cdot A} = o$
$p_{X \cdot B} = b$	$p_{Y \cdot B} = b'$
$p_{X \cdot C} = c$	$p_{Y \cdot C} = c'$
$p_{X \cdot D} = d$	$p_{Y \cdot D} = d'$
$p_{X \cdot E} = o$	$p_{Y \cdot E} = e'$

B, C, and D are assumed to be independent of each other—that is, $r_{BC} = 0$, etc.

Hence $p_{X \cdot B} = r_{XB}$, etc.

$$ {}_{B}r_{XY} = \frac{r_{XY} - bb'}{\sqrt{(1-b^2)\,(1-b'^2)}} $$

$$ {}_{CB}r_{XY} = \frac{{}_{B}r_{XY} - {}_{B}r_{XC}\,{}_{B}r_{YC}}{\sqrt{(1-{}_{B}r^2_{XC})\,(1-{}_{B}r^2_{YC})}} = \frac{r_{XY} - bb' - cc'}{\sqrt{(1-b^2-c^2)(1-b'^2-c'^2)}}. $$

When all common causes have been made constant, ${}_{DCB}r_{XY} = 0$

$$ r_{XY} = bb' + cc' + dd' = \Sigma p_{X \cdot B} p_{Y \cdot B}. $$

Thus, in those cases in which the causes are independent of each other, the correlation between two variables equals the sum of the products of the pairs of path coefficients which connect the two variables with each common cause. An illustration of the use of this principle was given in an earlier paper (*8*) in analyzing the nature of size factors in rabbits.

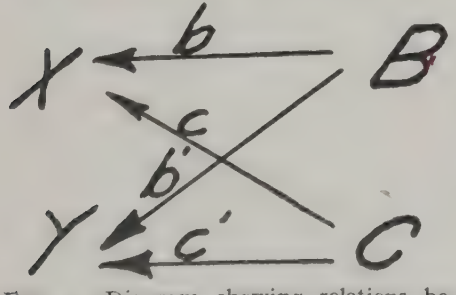


FIG. 3.—Diagram showing relations between two variables, X and Y, whose values are completely determined by common causes, B and C, which are independent of each other.

It may be deduced from the foregoing formula that two variables may even be completely determined by the same factors and yet be uncorrelated with each other. Let variation of X be completely determined by factors B and C, the path coefficients being b and c, respectively. Let Y be completely determined by the same factors, the path coefficients being b' and c' (fig. 3). Then $r_{XY} = bb' + cc'$. The condition

under which r_{XY} may equal zero is evidently that $bb' = -cc'$. An example may be found in the absence of correlation between the sum and difference of pairs of numbers picked at random from a table.

In many cases a small actual correlation between variables will be found on analysis to be the resultant of a balancing of very much more important but opposed paths of influence leading from common causes.

SYSTEMS OF CORRELATED CAUSES

The discussion up to this point has dealt wholly with causes which act independently of each other. It is necessary to consider the effects of correlation among the causes.

Let us consider the sum of two correlated variables (fig. 4).

$$\text{Let } X = M + N$$
$$\sigma^2_X = \sigma^2_M + \sigma^2_N + 2\sigma_M\sigma_N r_{MN}.$$

We have defined $\sigma_{X \cdot M}$ as the standard deviation of X when factors other than M are constant, but M varies as much as before. The latter qualification is important in the present case, since the making of N constant tends to reduce the variation of M, reducing σ_M to $\sigma_M\sqrt{1 - r^2_{MN}}$. The definition of $\sigma_{X \cdot M}$ implies that not only is N made constant but that there is such a readjustment among the more remote causes, A, B, and C, that σ_M is unchanged. Under the definition it is evident that in this case $\sigma_{X \cdot M} = \sigma_M$ and $\sigma_{X \cdot N} = \sigma_N$. Thus $p_{X \cdot M} = \frac{\sigma_M}{\sigma_X}$ and $p_{X \cdot N} = \frac{\sigma_N}{\sigma_X}$.

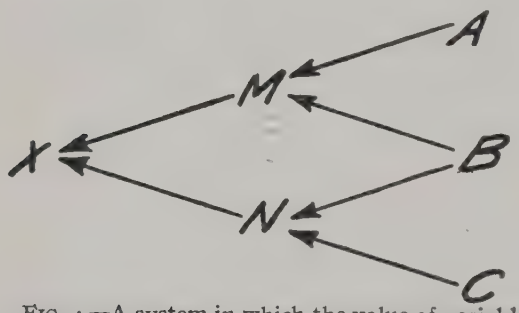


FIG. 4.—A system in which the value of variable X is completely determined by causes M and N, which are correlated with each other.

In attempting to find the degrees of determination of X by M and N we meet a difficulty somewhat similar to that met in the case of non-additive factors. The squared standard deviation is made up in part of elements due wholly to M and N, respectively, but in part to a portion which can not be divided between them. The term $2\sigma_M\sigma_N r_{MN}$ is due solely to the fact that the variations of X, which M and N determine, tend to be in the same direction and so have greater effect than if variations M and N were combined at random. It seems best to define $d_{X \cdot M}$ as the degree of determination of X due to M alone. Thus $d_{X \cdot M} = \frac{\sigma^2_M}{\sigma^2_X}$, $d_{X \cdot N} = \frac{\sigma^2_N}{\sigma^2_X}$. The remaining term may be considered as determination by M and N jointly and may be written $d_{X \cdot \overline{MN}} = 2p_{X \cdot M}p_{X \cdot N}r_{MN}$.

These rules can be extended at once to the sums of more than two variables, to sums of multiples of variables, and hence, as before, to

linear relations of cause and effect in which the influence of the causes is combined additively. It is also easy to show that the formulae apply approximately for multiplying factors.

Summing up, $p_{X\cdot M} = \sqrt{d_{X\cdot M}} = \frac{\sigma_{X\cdot M}}{\sigma_X}$

$$\Sigma d_{X\cdot M} + 2\Sigma p_{X\cdot M} p_{X\cdot N} r_{MN} = 1.$$

The next problem is to find the degree of determination of X by a factor such as B, which is connected with X by more than one path (fig. 5).

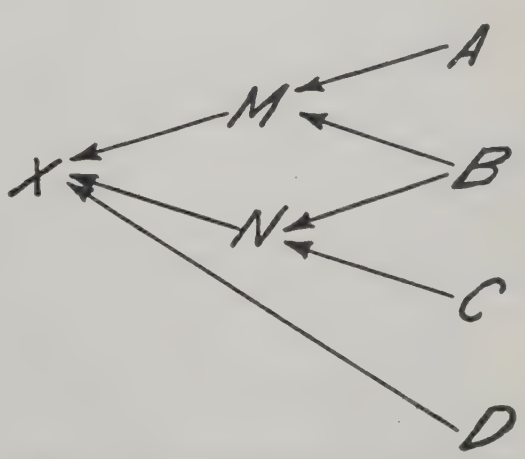


FIG. 5.—A system in which the value of X is affected by a factor, B, along two different paths, BMX and BNX.

Assume that A, B, C, and D are independent and completely determine X. $d_{X\cdot A} + d_{X\cdot B} + d_{X\cdot C} + d_{X\cdot D} = 1$. But also $d_{X\cdot M} + d_{X\cdot N} + 2p_{X\cdot M}p_{X\cdot N}r_{MN} + d_{X\cdot D} = 1$.

$d_{X\cdot B} = d_{X\cdot M} - d_{X\cdot A} + d_{X\cdot N} - d_{X\cdot C} + 2p_{X\cdot M}p_{X\cdot N}p_{M\cdot B}p_{N\cdot B}$, remembering that $r_{MN} = p_{M\cdot B}p_{N\cdot B}$.

Since $d_{M\cdot A} + d_{M\cdot B} = 1$, etc., we have $d_{X\cdot M} = d_{X\cdot M}d_{M\cdot A} + d_{X\cdot M}d_{M\cdot B} = d_{X\cdot A} + d_{X\cdot M}d_{M\cdot B}$, and $d_{X\cdot N} = d_{X\cdot C} + d_{X\cdot N}d_{N\cdot B}$.

Therefore
$$\begin{aligned} d_{X\cdot B} &= d_{X\cdot M}d_{M\cdot B} + d_{X\cdot N}d_{N\cdot B} + 2p_{X\cdot M}p_{X\cdot N}p_{M\cdot B}p_{N\cdot B} \\ &= p^2_{X\cdot M}p^2_{M\cdot B} + p^2_{X\cdot N}p^2_{N\cdot B} + 2p_{X\cdot M}p_{X\cdot N}p_{M\cdot B}p_{N\,B} \\ &= (p_{X\cdot M}p_{M\cdot B} + p_{X\cdot N}p_{N\cdot B})^2 \\ p_{X\cdot B} &= p_{X\cdot M}p_{M\cdot B} + p_{X\cdot N}p_{N\cdot B}. \end{aligned}$$

These results are easily extended to cases in which B acts on X through any number of causes. If a path coefficient is assigned to each component path, the combined path coefficient for all paths connecting an effect with a remote cause equals the sum of the products of the path coefficients along all the paths. Since B is independent of A, C, and D, $r_{X\cdot B} = p_{X\cdot B} = p_{X\cdot M}p_{M\cdot B} + p_{X\cdot N}p_{N\cdot B}$.

GENERAL FORMULA

We are now in a position to express the correlation between any two variables in terms of path coefficients. Let X and Y be two variables which are affected by correlated causes M and N. Represent the various path coefficients by small letters as in the diagram. Let A, B, and C be hypothetical remote causes which are independent of each other (fig. 6).

$$\begin{aligned} r_{XY} &= p_{X\cdot A}p_{Y\cdot A} + p_{X\cdot B}p_{Y\cdot B} + p_{\cdot C}p_{Y\cdot C} \\ &= mam'a + (mb + nb')(m'b + n'b') + ncn'c \\ &= mm' + mbb'n' + nn' + nb'bm'. \end{aligned}$$

Thus, the correlation between two variables is equal to the sum of the products of the chains of path coefficients along all of the paths by which they are connected.

If we know only the effects, X and Y, and correlated causes, such as M and N, it will be well to substitute r_{MN} for bb' in the foregoing formula.

$$r_{XY} = p_{X\cdot M}p_{Y\cdot M} + p_{X\cdot M}r_{MN}p_{Y\cdot N} + p_{X\cdot N}p_{Y\cdot N} + p_{X\cdot N}r_{MN}p_{Y\cdot M}.$$

We have reached a general formula expressing correlation in terms of path coefficients. This is not the order in which knowledge of the coefficients must be obtained, but, nevertheless, by means of simultaneous equations the values of the path coefficients in a system can often be calculated from the known correlations. Additional equations are furnished by the principle that the sum of the degrees of determination must

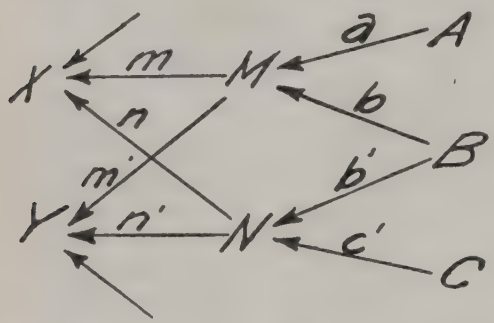


FIG. 6. Diagram showing relations between two variables, X and Y, whose values are determined in part by common causes, M and N, which are correlated with each other.

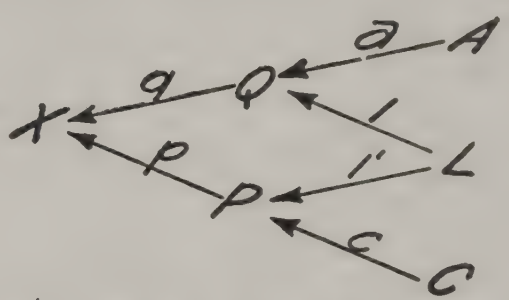


FIG. 7.—Simplified diagram of factors which determine birth weight in guinea pigs.

equal unity. The fundamental equations can be written in general form as follows:

$$d_{X\cdot A} = p^2_{X\cdot A}$$

$$d_{X\cdot \overline{AB}} = 2p_{X\cdot A}p_{X\cdot B}r_{AB}$$

$$\Sigma d_{X\cdot A} + \Sigma d_{X\cdot \overline{AB}} = 1$$

$$r_{XY} = \Sigma p_{X\cdot A}p_{Y\cdot A}.$$

APPLICATION TO BIRTH WEIGHT OF GUINEA PIGS

As a simple example, we may consider the factors which determine birth weight in guinea pigs (fig. 7).

Let X be birth weight; Q, prenatal growth curve; P, gestation period; L, size of litter; A, hereditary and environmental factors which determine Q, apart from size of litter; C, factors determining gestation period apart from size of litter.

For the sake of simplicity, it will be assumed that the interval between litters (if less than 75 days) accurately measures the gestation period

and that the variables are connected only by the paths shown above. In a certain stock of guinea pigs the following correlations were found:

Birth weight with interval, $r_{XP} = +0.5547$.

Birth weight with litter, $r_{XL} = -0.6578$.

Interval with litter, $r_{PL} = -0.4444$.

We are able to form three equations of type $r_{XY} = \Sigma p_{X \cdot A} p_{Y \cdot A}$ and three of type $\Sigma p^2_{X \cdot A} + 2\Sigma p_{X \cdot A} p_{X \cdot B} r_{AB} = 1$. These six equations will enable us to calculate six unknown quantities. The six path coefficients in the diagram in figure 7 can thus be calculated from the information given here, but no others.

The equations are as follows:

(1) $r_{XP} = +0.5547 = p + qll'$.

(2) $r_{XL} = -0.6578 = ql + pl'$.

(3) $r_{PL} = -0.4444 = l'$.

(4) $q^2 + p^2 + 2qpll' = 1$.

(5) $a^2 + l^2 = 1$.

(6) $l'^2 + c^2 = 1$.

From (3), $p_{P \cdot L} = l' = -0.4444$ $d_{P \cdot L} = l'^2$ $= 0.1975$
From (6), $p_{P \cdot C} = c = 0.8958$ $d_{P \cdot C} = c^2$ $= .8025$
1.0000

From (1) and (2), $p_{X \cdot P} = p = 0.3269$ $d_{X \cdot P} = p^2$ $= 0.1069$
$ql = -0.5125$ $d_{X \cdot Q} = q^2$ $= .7442$
From (4), $p_{X \cdot Q} = q = 0.8627$ $d_{X \cdot \overline{PQ}} = 2pqll'$ $= .1489$
1.0000

$p_{Q \cdot L} = l = -0.5941$ $d_{Q \cdot L} = l^2$ $= 0.3530$
$p_{Q \cdot A} = a = 0.8044$ $d_{Q \cdot A} = a^2$ $= .6470$
1.0000

$d_{X \cdot Q \cdot L} = q^2 l^2$ $= 0.2627$
$d_{X \cdot P \cdot L} = p^2 l'^2$ $= .0211$
$d_{X \cdot \overline{PQ} \cdot L} = 2pqll'$ $= .1489$
$d_{X \cdot L} = (ql + pl')^2$ $= .4327$

$d_{X \cdot A} = q^2 a^2$ $= .4815$
$d_{X \cdot C} = p^2 c^2$ $= .0858$
1.0000

Assuming that the diagrams in figures 7, 8, and 9 accurately represent the causal relations, it appears that birth weight is determined to a very much greater extent by variations in the rate of growth of the foetuses than by variations in the length of the gestation period ($d_{X \cdot Q} = 0.74$, $d_{X \cdot P} = 0.11$). Size of litter has much more effect on birth weight by reducing the rate of growth of the foetuses than by causing early parturition ($d_{X.Q.L} = 0.26$, $d_{X.P.L} = 0.02$). The difference in birth weight caused by a difference of a day in gestation period can be calculated from the path coefficient and the standard deviations by the formula for path regression, $p.\ reg_{X \cdot P} = p_{X \cdot P} \frac{\sigma_X}{\sigma_P}$. The result, 3.34 gm. per day, should measure the average rate of growth just preceding parturition. The actual regression, 5.66 gm. per day of delay in parturition, is larger because a long gestation period means not merely a longer time for growth but also, in general, a smaller litter and hence more rapid growth.

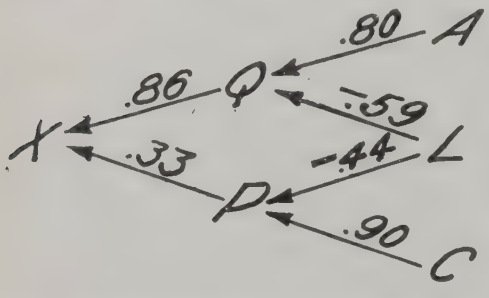


FIG. 8.—Path coefficients measuring the relations between birth rate (X), rate of growth (Q), gestation period (P), size of litter (L), and other causes (A, C).

On introducing other data the analysis can be carried much farther. There are other paths of influence which should be recognized, positive paths connecting A, C, and L, representing the favorable effects of good health in the dam on rate of growth, gestation period, and size of litter, and a negative path from Q to P to represent the tendency of rapid growth to induce early parturition. The relations between the observed interval between litters and the actual gestation period should also be considered. The results presented here are thus intended merely to furnish a simple illustration of the method. A more complete analysis of the relations among the factors which affect birth weight and later growth will be presented in a later paper.

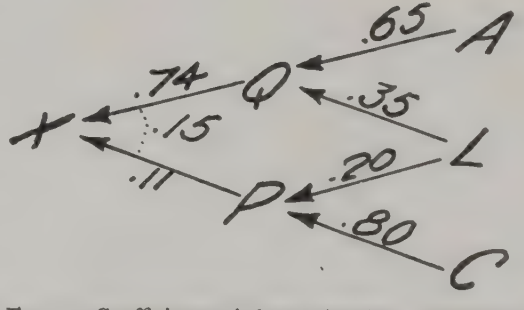


FIG. 9.—Coefficients of determination. Symbols as in figure 7.

DETERMINATION IN TERMS OF CORRELATION

Having obtained a formula for correlation in terms of determination, the question arises whether the converse is possible. For a special class of cases such a formula is easily obtained.

For a single cause and effect the required formula is merely $d_{X \cdot A} = r^2_{XA}$ (fig. 10).

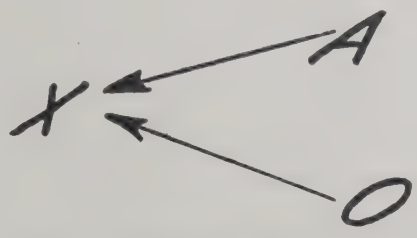


FIG. 10.—Effect and one known cause.

The degree of determination by residual factors; that is, $d_{X \cdot O}$, is thus $1 - r^2_{XA}$.

If two causes are known, and the degree of correlation between them, we have (fig. 11)—

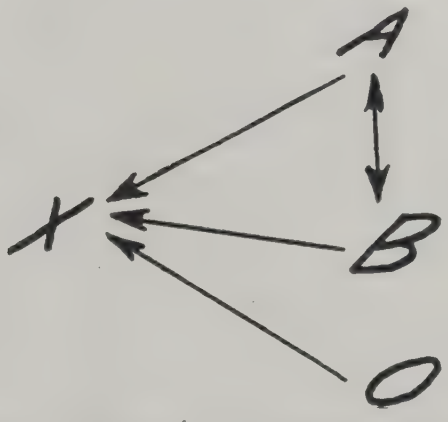


FIG. 11.—Effect and two correlated known causes.

$$ {}_B r^2_{XA} + {}_B r^2_{XO} = 1 $$

$$ \frac{(r_{XA} - r_{XB} r_{AB})^2}{(1 - r^2_{XB})(1 - r^2_{AB})} = 1 - \frac{r^2_{XO}}{1 - r^2_{XB}} $$

$$ r^2_{XO} = d_{X \cdot O} = \frac{1 - r^2_{XA} - r^2_{XB} - r^2_{AB} + 2 r_{XA} r_{XB} r_{AB}}{1 - r^2_{AB}}. $$

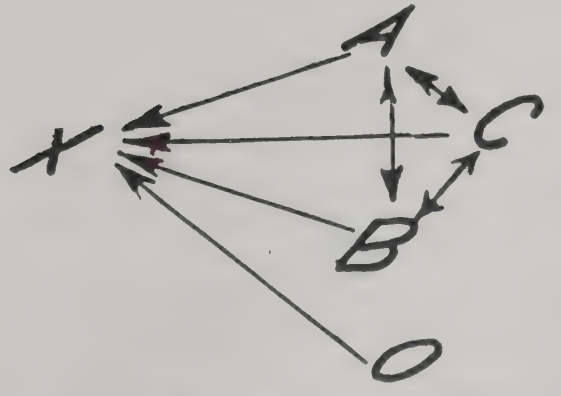


FIG. 12.—Effect and three correlated known causes.

If three causes and their correlations are known (fig. 12), we have ${}_{CB} r^2_{XA} + {}_{CB} r^2_{XO} = 1$, from which

$$ r^2_{XO} = d_{X \cdot O} = \frac{1 - \Sigma r^2_{XA} + 2 \Sigma r_{XA} r_{AB} r_{BX} - 2 \Sigma r_{XA} r_{AB} r_{BC} r_{CX} + \Sigma r^2_{XA} r^2_{BC}}{1 - r^2_{AB} - r^2_{AC} - r^2_{BC} + 2 r_{AC} r_{CB} r_{BA}}. $$

In this expression Σr^2_{XA} means the sum of squares of the six known correlations. $\Sigma r_{XA} r_{AB} r_{BX}$ means the sum of the products of the groups of three correlations, corresponding to the sides of triangles. There are four of these triangles, XAC, XAB, XCB, ABC. $\Sigma r_{XA} r_{AB} r_{BC} r_{CX}$ means the sum of the three products of the groups of correlations which are arranged in closed quadrilaterals, and $\Sigma r^2_{XA} r^2_{BC}$ means the sum of the product of squared correlations in pairs which involve no common variable ($r^2_{XA} r^2_{BC}$, $r^2_{XC} r^2_{AB}$, $r^2_{XB} r^2_{AC}$) (fig. 13).

The formula for four known causes is easily found by a continuation of the methods used to find the others if attention is paid to the symmetry of the expressions. Since, however, this formula, as well as that just given for the case of three causes, is somewhat cumbersome, it will be convenient to use a more condensed notation. $\phi(XABC \ldots)$ may be used for a function involving all possible correlations among the variables $(XABC \ldots)$. In the definitions Σr^2 means the sum of the squares of all correlations; $\Sigma r^2 r^2$, the sum of the product of all pairs of squared correlations which involve no variables in common; Σrrr, $\Sigma rrrr$, and $\Sigma rrrrr$ are the sums of the products of all groups of correlations which, represented by paths, form closed figures, triangles, quadrilaterals, and pentagons, respectively. $\Sigma r^2 rrr$ is the sum of the products made by multiplying each triangle of correlations in the sense above by the second power of those correlations which do not involve any of the variables in the triangle. The number of terms of each kind is given above the brace, where it is more than one.

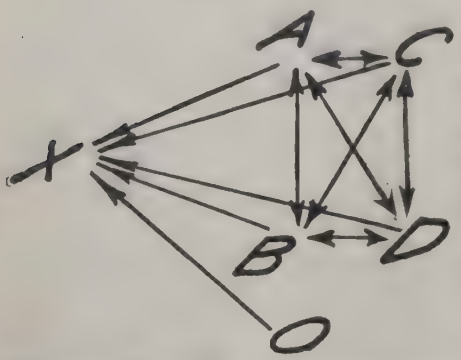


FIG. 13.—Effect and four correlated known causes.

$$\phi(AB) = 1 - r^2 \text{ (2 terms)}.$$

$$\phi(ABC) = 1 - \overbrace{\Sigma r^2}^{3} + 2\Sigma rrr \text{ (5 terms)}.$$

$$\phi(ABCD) = 1 - \overbrace{\Sigma r^2}^{6} + \overbrace{2\Sigma rrr}^{4} - \overbrace{2\Sigma rrrr}^{3} + \overbrace{\Sigma r^2 r^2}^{3} \text{ (17 terms)}.$$

$$\phi(ABCDE) = 1 - \overbrace{\Sigma r^2}^{10} + \overbrace{2\Sigma rrr}^{10} - \overbrace{2\Sigma rrrr}^{15} + \overbrace{2\Sigma rrrrr}^{12} + \overbrace{\Sigma r^2 r^2}^{15} - \overbrace{2\Sigma r^2 rrr}^{10} \text{ (73 terms)}.$$

The formulae for degree of determination by residual factors may be written as follows:

$d_{X\cdot O}=\phi(XA)$ in system XA.

$d_{X\cdot O}=\dfrac{\phi(XAB)}{\phi(AB)}$ in system XAB.

$d_{X\cdot O}=\dfrac{\phi(XABC)}{\phi(ABC)}$ in system $XABC$.

$d_{X\cdot O}=\dfrac{\phi(XABCD)}{\phi(ABCD)}$ in system $XABCD$.

The degree of determination by the known causes is now easily calculated. When all causes of variation in X are constant except A, variation of X is measured by ${}_{O\cdots CB}\sigma_X$ and variation of A is measured by ${}_{O\cdots CB}\sigma_A$, writing the constant factors as subscripts to the left. Assuming that the relation between A and X is linear, the deviation of X determined by a unit deviation of A should be constant, whatever the amount of variation in A. Thus:

$$p_{X\cdot A}\frac{\sigma_X}{\sigma_A}=\frac{\sigma_{X\cdot A}}{\sigma_A}=\frac{{}_{O\cdots CB}\sigma_X}{{}_{O\cdots CB}\sigma_A}.$$

In the case of the residual factor O, assumed to be independent of the known factors A, B, C, etc., ${}_{\cdots CBA}\sigma_O=\sigma_O$,
and we have $\sigma_{X\cdot O}={}_{\cdots CBA}\sigma_X$

$$d_{X\cdot O}=\frac{\phi(XABC\ldots)}{\phi(ABC\ldots)}=\frac{\sigma^2{}_{X\cdot O}}{\sigma^2{}_X}=\frac{{}_{\cdots CBA}\sigma^2{}_X}{\sigma^2{}_X}.$$

Thus:

$${}_{\cdots CBA}\sigma^2{}_X=\frac{\phi(XABC\ldots)}{\phi(ABC\ldots)}\sigma^2{}_X.$$

This should be the general formula for the squared standard deviation with a number of constant factors.
Hence:

$$\frac{\sigma^2{}_{X\cdot A}}{\sigma^2{}_A}=\frac{\phi(XBC\ldots O)}{\phi(BC\ldots O)}\sigma^2{}_X\Big/\frac{\phi(ABC\ldots O)}{\phi(BC\ldots O)}\sigma^2{}_A$$

$$\sigma^2{}_{X\cdot A}=\frac{\phi(XBC\ldots O)}{\phi(ABC\ldots O)}\sigma^2{}_X$$

$$p_{X\cdot A}=\sqrt{\frac{\phi(XBC\ldots O)}{\phi(ABC\ldots O)}}$$

$$d_{X\cdot A}=\frac{\phi(XBC\ldots O)}{\phi(ABC\ldots O)}=\frac{\phi(XBC\ldots)-d_{X\cdot O}\phi(BC\ldots)}{\phi(ABC\ldots)}.$$

The general formula for partial correlation can easily be expressed in the present terminology.

$$_{DCBA}\sigma^2_X = {}_{DCB}\sigma^2_X(1 - {}_{DCB}r^2_{XA})$$

$${}_{DCB}r^2_{XA} = 1 - \frac{{}_{DCBA}\sigma^2_X}{{}_{DCB}\sigma^2_X} = 1 - \frac{\phi(XABCD)\phi(BCD)}{\phi(ABCD)\phi(XBCD)}.$$

In some cases it may be of interest to find the degree of determination when a number of factors not in the direct path between cause and effect are assumed constant.

$${}_{UTS}d_{X\cdot A} = \frac{{}_{UTS}\sigma^2_{X\cdot A}}{{}_{UTS}\sigma^2_X} = \frac{({}_{O\ldots UTS\ldots CB}\sigma^2_X)({}_{UTS}\sigma^2_A)}{({}_{O\ldots UTS\ldots CB}\sigma^2_A)({}_{UTS}\sigma^2_X)}$$

$$= \frac{\phi(XBC\ldots STU\ldots O)\phi(ASTU)}{\phi(ABC\ldots STU)\phi(XSTU)}.$$

RELATION TO MULTIPLE CORRELATION

The expressions defined as $\phi(XABC\ldots)$, etc., suggest the expansion of determinants. It is in fact easy to show that $\phi(XABC\ldots N) = \Delta$.

Where

$$\Delta = \begin{vmatrix} 1 & r_{XA} & r_{XB} & \cdot & \cdot & r_{XN} \\ r_{AX} & 1 & r_{AB} & \cdot & \cdot & r_{AN} \\ r_{BX} & r_{BA} & 1 & \cdot & \cdot & r_{BN} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ r_{NX} & r_{NA} & r_{NB} & \cdot & \cdot & 1 \end{vmatrix}$$

The formula for Pearson's coefficient of multiple correlation has already been given, $R_{X(ABCO)} = \sqrt{1 - \frac{\Delta}{\Delta_{XX}}}$ where Δ_{XX} is the minor made by deleting row X, column X.

Evidently in this class of cases the coefficient of determination degenerates into a function of the coefficient of multiple correlation. For the degree of determination by residual factors we have

$$d_{X\cdot O} = \frac{\phi(XABC\ldots)}{\phi(ABC\ldots)} = \frac{\Delta}{\Delta_{XX}} = 1 - R^2_{X(ABC\cdots)}$$

in agreement with Pearson's results.

For the degree of determination by a known factor we have

$$d_{X\cdot A} = \frac{\phi(XBC\ldots O)}{\phi(ABC\ldots O)} = \frac{\phi(XBC\ldots) - d_{X\cdot O}\phi(BC\ldots)}{\phi(ABC\ldots)} = \frac{\Delta_{AA}\Delta_{XX} - \Delta\Delta_{AAXX}}{\Delta^2_{XX}}$$

$$= \frac{\Delta^2_{XA}}{\Delta^2_{XX}}.$$

$$p_{X\cdot A} = \frac{\Delta_{XA}}{\Delta_{XX}}.$$

The last formula brings out the close relation between the path coefficients and multiple regression. As already noted, the most probable deviation of X for known deviations of A, B, C, etc., is given by the formula

$$\frac{X'}{\sigma_X} = \frac{\Delta_{XA}}{\Delta_{XX}}\frac{A'}{\sigma_A} + \frac{\Delta_{XB}}{\Delta_{XX}}\frac{B'}{\sigma_B} \cdots = p_{X \cdot A}\frac{A'}{\sigma_A} + p_{X \cdot B}\frac{B'}{\sigma_B} \cdots$$

As already stated, Pearson's coefficients of multiple correlation and regression were not devised especially for the analysis of causal relations. The formula for multiple regression, for example, gives the most probable value of one of the variates for given values of the others regardless of causal relations. In cases in which all the correlations are known in a system including an effect and a number of causes the method can be used to find the path coefficients and the degrees of determination of the effect by each cause in the sense used in this paper. Such cases in which the direct methods can be used are, however, relatively uncommon. Where the system of paths of influence is at all complex, involving perhaps hypothetical factors, the causal relations can be analyzed only by the indirect method of expressing the known correlations in terms of the unknown path coefficients, making the sums of the degrees of determination unity and solving the simultaneous equations.

PART II. APPLICATION TO THE TRANSPIRATION OF PLANTS

A large body of experimental data on the factors which affect the rate of transpiration in plants has been published by Briggs and Shantz (*2*). These data are well adapted for use in illustrating the methods of analyzing causal relations presented in part I of this paper.

The experiments which are used in this paper were conducted at Akron, Colo., in 1914. A variety of crop plants were grown in sealed pots. The total transpiration was measured each day. Among the environmental factors studied were the total solar radiation during the day, the wind velocity, the air temperature (in the shade), the rate of evaporation from a shallow tank, and the wet-bulb depression (sheltered from sun but not wind). The correlations between the daily transpiration of each kind of plant and the integrated values of the environmental factors were published by Briggs and Shantz. In order to avoid the effect of seasonal change in the plants, the logarithms of the ratios of the transpiration on succeeding days were correlated with similar figures for the various factors. The correlations between the various environmental factors for the 100 days from June 18 to September 25, 1914, have been calculated by the writer from the data presented by Briggs and Shantz. This period covers all the crop periods but is longer than most of them. None of the correlations appeared to depart much from linearity.

The daily averages, the standard deviations, and the correlations are given in Table I.

TABLE I.—*Daily averages, standard deviations, and correlations from experiments on transpiration in crop plants made by Briggs and Shantz at Akron, Colo., 1914*

CORRELATIONS

	Wind.	Radiation.	Temperature.	Wet-bulb depression.	Evaporation.
Wind		−0.01 ±0.07	−0.02 ±0.07	0.28 ±0.06	0.38 ±0.06
Radiation	−0.01 ±0.07		.47 ± .05	.48 ± .05	.68 ± .04
Temperature	− .02 ± .07	.47 ± .05		.59 ± .05	.56 ± .05
Wet-bulb depression	.28 ± .06	.48 ± .05	.59 ± .05		.83 ± .02
Evaporation	.38 ± .06	.68 ± .04	.56 ± .05	.83 ± .02	
Small grains[a]	.22 ± .04	.65 ± .03	.71 ± .02	.88 ± .01	.87 ± .02
Rye	.19 ± .10	.65 ± .06	.73 ± .05	.94 ± .01	.91 ± .02
Sorghum, millet[b]	.218± .041	.570± .030	.653± .026	.788± .018	.713± .021
Sudan grass (in inclosure)	.52 ± .07	.55 ± .06	.84 ± .03	.83 ± .03	.93 ± .01
Sudan grass (in open)	.32 ± .08	.52 ± .07	.81 ± .03	.85 ± .03	.82 ± .03
Dent corn	.28 ± .08	[e].52 ± .06	.71 ± .04	.81 ± .03	.79 ± .03
Algerian corn	.33 ± .09	.62 ± .06	.79 ± .04	.88 ± .02	.85 ± .03
Cowpea, lupine[c]	.335± .057	.570± .042	.675± .035	.785± .025	.775± .025
Alfalfa[d]	.290± .035	.430± .030	.495± .029	.700± .019	.705± .019
Amaranthus	.04 ± .10	.40 ± .09	.45 ± .08	.60 ± .07	.56 ± .06

	Mean.	σ
Evaporation (shallow tank) (kilograms per square meter)	9.70	2.76
Integrated radiation (calories per square centimeter)	753	134
Air temperature, integrated mean (degrees Centigrade)	20.10	3.48
Integrated wet-bulb depression (hour degrees, Centigrade)	143	58
Wind velocity (miles per hour)	5.54	2.24

[a] Averages of six similar correlations involving Kubanka and Galgalos wheat, Swedish Select and Burt oats, Hannchen barley, and spring rye. The last, having on the whole the largest correlations, is also given separately.

[b] Averages of four correlations, Minnesota Amber and Dakota Amber sorghum and Kursh and Siberian Millet. These correlations were all very similar.

[c] Average of the similar correlations for cowpeas and lupine.

[d] Average of four tests with alfalfa.

[e] Published as +0.80, which seems too large. Recalculation gives +0.52.

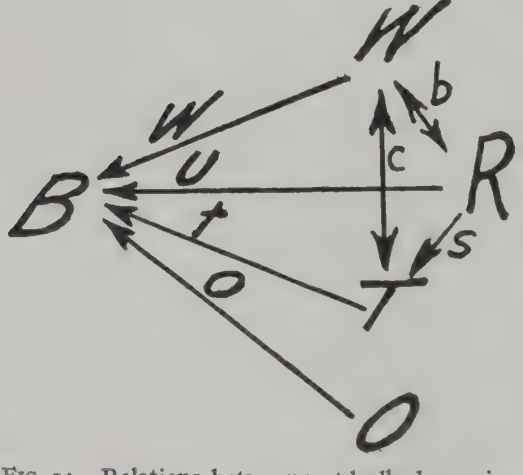


FIG. 14.—Relations between wet-bulb depression (*B*), wind velocity (*W*), radiation (*R*), and temperature (*T*) as assumed for direct analysis.

It will be interesting first to compare the direct and indirect methods of calculating path coefficients and coefficients of determination. Let us consider the relations of wet-bulb depression (*B*) to temperature (*T*), wind velocity (*W*), and radiation (*R*). Since the direct methods are only applicable in systems in which each variable is connected with every other variable, the diagram of relations is as shown in figure 14. Outstanding factors, independent of *W*, *R*, and *T* are represented by *O*.

INDIRECT METHOD

Six equations can be formed, expressing the six known correlations in terms of the unknown path coefficients. A seventh equation represents the complete determination of B by W, R, T, and O.

(1) $r_{BW} = 0.28 = w + t(c + bs) + ub.$
(2) $r_{BR} = .48 = wb + ts + u.$
(3) $r_{BT} = .59 = w(c + bs) + t + us.$
(4) $r_{WR} = -.01 = b.$
(5) $r_{WT} = -.02 = c + bs.$
(6) $r_{RT} = .47 = s.$
(7) $o^2 + w^2 + t^2 + u^2 + 2wt(c + bs) + 2wub + 2uts = 1.$

The values of b and s are given directly from equations (4) and (6), and the value of c ($= -0.0153$) can then be obtained from (5). The solution of (1), (2), and (3) gives $w = 0.2921$, $t = 0.4735$, and $u = 0.2604$. Finally, from (7) we obtain $o^2 = 0.5138$ as the degree of determination by outstanding factors.

$d_{B\cdot O} = o^2$	=	0.5138	
$d_{B\cdot W} = w^2$	=	.0853	$p_{B\cdot W} = w = 0.2921$
$d_{B\cdot T} = t^2$	=	.2242	$p_{B\cdot T} = t = .4735$
$d_{B\cdot R} = u^2$	=	.0678	$p_{B\cdot R} = u = .2604$
$d_{B\cdot \overline{WT}} = 2wt(c + bs)$	= −	.0055	
$d_{B\cdot \overline{WR}} = 2wub$	= −	.0015	
$d_{B\cdot \overline{RT}} = 2uts$	=	.1159	
		1.0000	

DIRECT METHODS

According to the formulae given in part I we have—

$$d_{B\cdot O} = \frac{\phi(BWRT)}{\phi(WRT)}$$

$$d_{B\cdot W} = \frac{\phi(BRT) - d_{B\cdot O}\phi(RT)}{\phi(WRT)}$$

$$d_{B\cdot R} = \frac{\phi(BWT) - d_{B\cdot O}\phi(WT)}{\phi(WRT)}$$

$$d_{B\cdot T} = \frac{\phi(BRW) - d_{B\cdot O}\phi(RW)}{\phi(WRT)}$$

where

$$\begin{aligned}\phi(BWRT) = 1 &- r^2_{BW} + 2r_{BW}r_{WR}r_{RB} - 2r_{BW}r_{WR}r_{RT}r_{TB} + r^2_{BW}r^2_{RT} \\ &- r^2_{BR} + 2r_{BW}r_{WT}r_{TB} - 2r_{BW}r_{WT}r_{TR}r_{RB} + r^2_{BR}r^2_{WT} \\ &- r^2_{BT} + 2r_{BR}r_{RT}r_{TB} - 2r_{BR}r_{RW}r_{WT}r_{TB} + r^2_{BT}r^2_{WR} \\ &- r^2_{WR} + 2r_{WR}r_{RT}r_{TW} \\ &- r^2_{WT} \\ &- r^2_{RT}\end{aligned}$$

$\phi(WRT) = 1 - r^2_{WR} - r^2_{WT} - r^2_{RT} + 2r_{WR}r_{RT}r_{TW}$
$\phi(BWR)$, etc., are analogous to $\phi(WRT)$
$\phi(RT) = 1 - r^2_{TR}$ $\phi(WT)$, etc., are analogous to $\phi(RT)$.

By substitution of the correlations in these formulae the following results are obtained:

$$\phi(BWRT) = 0.4002$$

$\phi(BWR) = .6884$	$\phi(BW) = 0.9216$	$\phi(WR) = 0.9999$
$\phi(BWT) = .5665$	$\phi(BR) = .7696$	$\phi(WT) = .9996$
$\phi(BRT) = .4668$	$\phi(BT) = .6519$	$\phi(RT) = .7791$
$\phi(WRT) = .7788$		

These give values of the coefficients of determination identical with those given by the indirect method.

This method, as was shown in part I, is essentially the same as Pearson's method of calculating multiple regression.

Let $\Delta = \begin{vmatrix} 1 & r_{BR} & r_{BT} & r_{BW} \\ r_{RB} & 1 & r_{RT} & r_{RW} \\ r_{TB} & r_{TR} & 1 & r_{TW} \\ r_{WB} & r_{WR} & r_{WT} & 1 \end{vmatrix} = \begin{vmatrix} 1 & 0.48 & 0.59 & 0.28 \\ .48 & 1 & .47 & -.01 \\ .59 & .47 & 1 & -.02 \\ .28 & -.01 & -.02 & 1 \end{vmatrix} = 0.4002$

Let $\Delta_{BB} = \Delta$ with column B, row B, deleted.

$\Delta_{BB} = 0.7788$, $\Delta_{BR} = 0.2028$, $\Delta_{BT} = 0.3687$, $\Delta_{BW} = 0.2275$

$$p_{B\cdot W} = \frac{\Delta_{BW}}{\Delta_{BB}} = 0.2921 \qquad d_{B\cdot O} = \frac{\Delta}{\Delta_{BB}} = 0.5139$$

$$p_{B\cdot R} = \frac{\Delta_{BR}}{\Delta_{BB}} = 0.2604$$

$$p_{B\cdot T} = \frac{\Delta_{BT}}{\Delta_{BB}} = 0.4735.$$

These values are identical with those obtained by the preceding methods.

It will be seen that the first method, while apparently less direct than the others, is really less laborious. The solution of three simultaneous equations requires merely the evaluation of a determinant of the third order instead of one of the fourth order, as in the last method. The expression $\phi(BWRT)$ in the second method is, of course, merely an expansion of the same determinant of the fourth order as that used in the last. The indirect method, moreover, gives more insight into the processes followed than the others in which there is a substitution in what appear to be arbitrary formulae. In line with this last point, the indirect method is more flexible in that it can be used to test out the consequences of any assumed relation among the factors.

ANALYSIS OF CAUSAL RELATIONS

In attempting to interpret the present results in terms of causation, we see at once that the scheme of relations chosen is not a very satisfactory one. The wet-bulb depression was measured under shelter. Consequently the coefficient of determination, $d_{B\cdot R} = 0.0678$, can not measure

the degree of direct determination by radiation, but determination by some factor other than wind or temperature with which radiation is correlated.

One should not attempt to apply in general a causal interpretation to solutions by the direct methods. In these cases, determination can usually be used only in the sense in which it can be said that knowledge of the effect determines the probable value of the cause. This is the sense in which Pearson's formula for multiple regression must be interpreted. If W', T', and R' are given deviations of wind, temperature, and radiation from their mean values, the most probable value of the wet-bulb depression, B', is given by the following formula:

$$\frac{B'}{\sigma_B} = \frac{W'}{\sigma_W} p_{B\cdot W} + \frac{R'}{\sigma_R} p_{B\cdot R} + \frac{T'}{\sigma_T} p_{B\cdot T}.$$

This formula can only be used for conditions which are similar to those for which the values of the path coefficients were calculated. If path coefficients were calculated in a system which truly represented the causal relations, the formula would give the value of the wet-bulb depression under any set of conditions in so far as it is determined by the factors considered.

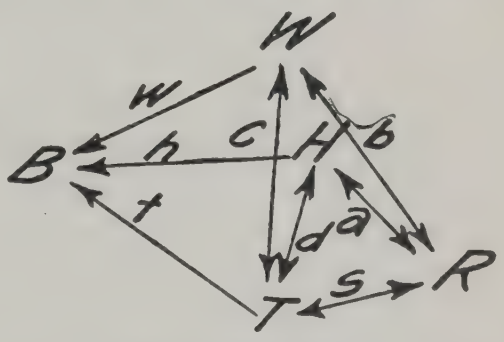


FIG. 15.—Relations between factors of figure 14 and absolute humidity (H) expressing causal relations better than figure 14 but adapted only to indirect analysis.

The causal factors which actually determine wet-bulb depression are temperature, absolute humidity (H), and wind velocity (fig. 15). Radiation can be introduced into the scheme as a factor correlated with these causal factors. Wind velocity is correlated to such a very slight extent with temperature and radiation that its correlation with absolute humidity can probably be neglected without serious error. The relations between radiation, temperature, and absolute humidity are undoubtedly very complex. Radiation has a direct positive influence on temperature. Both radiation and temperature have positive effects on absolute humidity by increasing evaporation. Correlation between absolute humidity and temperature would be expected, because with reduced temperature the saturation point is reached at a lower absolute humidity and the excess moisture is precipitated. Increase in humidity, on the other hand, tends to reduce the radiation which reaches the earth, and directly or indirectly this has a negative influence on all three of the correlations.

There are not enough data to estimate the importance of all of these paths of influence. Even if we represent the complex of paths connecting H, R, and T merely by three correlations, the diagram has eight paths to solve. The six correlations between B, W, R, and T and the statement

in regard to complete determination of B by W, H, and T furnish only seven equations.

Fortunately, data are given in another paper by Briggs and Shantz (3) from which an eighth equation can be derived. In this paper the average value of each of the measured factors is given for each hour of the day. The cycle of changes in wet-bulb depression follows very closely the changes in temperature. In fact, there should be very little, if any, regular hourly cycle of changes in absolute humidity, so that the wet-bulb depression should be wholly determined by the temperature changes except for some influence of wind velocity.

Let $p_{B.T}=t$ be the path coefficient which measures the relative influence of temperature on wet-bulb depression in the variations from day to day. Let $p_{B.H}=h$, $p_{B.W}=w$, and let σ_T, σ_H, σ_W, and σ_B be the standard deviations of the daily differences in the various factors and in wet-bulb depression. Let $T'-T''$, etc., be the actual differences in temperature, etc., at certain times. The difference to be expected in wet-bulb depression, $B'-B''$, is as follows:

$$\frac{B'-B''}{\sigma_B}=\frac{T'-T''}{\sigma_T}t+\frac{W'-W''}{\sigma_W}w+\frac{H'-H''}{\sigma_H}h.$$

While t, w, and h are assumed to measure the relative influence of temperature, wind, and humidity in the variations from day to day, the foregoing formula should apply under any conditions, if t, w, and h were calculated from a system which represented truly causal relations. The expression $\frac{\sigma_B}{\sigma_T}t$ is shown in part I to give the change in wet-bulb depression (B) directly caused by a unit change in temperature. The relative importance of the various factors in determining the variations from hour to hour is very different from that from day to day, but the change in wet-bulb depression caused by unit changes in temperature, wind velocity, or absolute humidity should always be the same so long as the relations are substantially linear.

The greatest difference in temperature within an average day in the data was between 5 a. m. and 3 p. m. This is given as 32.7° F., or 18.167° C. The difference in wet-bulb depression between these hours was 21.8° F., or 12.111° C. The difference in average wind velocity was 2.5 miles per hour. The standard deviations of the daily variations have already been given. $\sigma_T=3.48$ day degrees C., $\sigma_B=58$ hour degrees C. integrated for 24 hours. This means 2.4167 degrees C. $\sigma_W=2.24$ miles per hour. We will assume that there is no difference in absolute humidity ($H'-H''=0$). Substituting those values in the formula for wet-bulb depression, we get

$$\frac{12.111}{2.4167}=\frac{18.167}{3.48}t+\frac{2.50}{2.24}w$$

$$5.0114=5.2204t+1.1161w.$$

We now have eight equations from which to find eight unknown path coefficients.

(1) $r_{BW} = 0.28 = w + tc.$
(2) $r_{BR} = .48 = ts + bw + ah.$
(3) $r_{BT} = .59 = t + dh + wc.$
(4) $r_{WR} = -.01 = b.$
(5) $r_{WT} = -.02 = c.$
(6) $r_{RT} = .47 = s.$
(7) $w^2 + h^2 + t^2 + 2wtc + 2htd = 1.$
(8) $5.0114 = 5.2204t + 1.1161w.$

Equations (4), (5), and (6) give b, c, and s directly. Solution of (1) and (8) gives $t = 0.8963$, $w = 0.2979$.

From (2) $ah = 0.0617$
From (7) $h^2 = .6570$, $h = -0.8105$, $a = -0.0761$
From (3) $dh = -.3003$, $d = .3706$
$r_{BH} = h + td = -0.4784.$

The coefficients of determination, the path coefficients, and the correlations are thus as follows:

$d_{B\cdot T} = 0.8034$	$p_{B\cdot T} = 0.8963$	$r_{BT} = 0.5900$
$d_{B\cdot H} = .6570$	$p_{B\cdot H} = -.8105$	$r_{BH} = -.4784$
$d_{B\cdot W} = .0888$	$p_{B\cdot W} = .2979$	$r_{BW} = .2800$
$d_{B\cdot \overline{HT}} = -.5384$		
$d_{B\cdot \overline{WT}} = -.0107$		$r_{HR} = -.0761$
1.0001		$r_{HT} = .3706$
		$r_{RT} = .4700.$

It turns out that the differences between different days in wet-bulb depressions are due to a somewhat greater extent to differences in temperature (0.80) than to absolute humidity (0.66). The variation in wet-bulb depression would be much greater were it not that these factors vary together but act on wet-bulb depression in opposite directions and so tend to balance each other ($d_{B\cdot \overline{HT}} = -0.54$). Temperature shows a rather strong positive correlation with absolute humidity (0.37) as well as with radiation (0.47), but the various paths of influence between radiation and absolute humidity almost balance each other ($r_{HR} = -0.08$).

These results can now be used in finding the relative importance of the various factors which determine evaporation or transpiration. In figure 16, X may represent either evaporation or the transpiration of any plant. Radiation must be considered as a direct causal factor in these cases.

The following four equations can be made with which to solve the path coefficients from W, H, R, and T to X:

$$\begin{aligned}
r_{XW} &= w' + t'c + u'b \\
r_{XT} &= w'c + t' + u's + h'd \\
r_{XR} &= w'b + t's + u' + h'a \\
r_{XB} &= w'r_{BW} + t'r_{BT} + u'r_{BR} + h'r_{BH}.
\end{aligned}$$

Substituting the values already found for a, b, c, d, w, h, t, and r_{BH}, we have

$$\begin{aligned}
r_{XW} &= +1.00w' - 0.02t' - 0.01u' \\
r_{XT} &= -\ .02w' + 1.00t' + \ .47u' + 0.3706h' \\
r_{XR} &= -\ .01w' + \ .47t' + 1.00u' - \ .0761h' \\
r_{XB} &= +\ .28w' + \ .59t' + \ .48u' - \ .4784h'.
\end{aligned}$$

The solution is as follows:

$$\begin{aligned}
w' = p_{X\cdot W} &= +0.9971r_{XW} + 0.0143r_{XT} - 0.0022r_{XR} + 0.0114r_{XB} \\
t' = p_{X\cdot T} &= -\ .2207r_{XW} + \ .8943r_{XT} - \ .8175r_{XR} + \ .8228r_{XB} \\
u' = p_{X\cdot R} &= +\ .1488r_{XW} - \ .3633r_{XT} + 1.4155r_{XR} - \ .5067r_{XB} \\
h' = p_{X\cdot H} &= +\ .4607r_{XW} + \ .7468r_{XT} + \ .4107r_{XR} - 1.5772r_{XB}.
\end{aligned}$$

It is merely necessary to substitute the values of the correlations of evaporation or transpiration with wind velocity, temperature, radiation, and wet-bulb depression, as given in Table I, to find the four path coefficients in each case. The results are given in Table II. These have all been checked by substitution in the fourth equation ($r_{XB} = +0.28w' + 0.59t' + 0.48u' - 0.4784h'$). The correlation between evaporation and the transpiration of any plant can be deduced from the formula $r_{XE} = w'r_{EW} + t'r_{ET} + u'r_{ER} + h'r_{EH}$. The correlations of evaporation with wind velocity, temperature, and radiation have been given in Table I as 0.38, 0.56, and 0.68, and that with humidity can be calculated by the formula $r_{EH} = p_{E\cdot H} + a\,p_{E\cdot R} + d\,p_{E\cdot T} = -0.2651$. Thus $r_{XE} = 0.38w' + 0.56t' + 0.68u' - 0.2651h'$. The calculated results in column 6 of Table II are compared with actual correlations between evaporation and transpiration in column 7. The correlation of evaporation with itself comes out 0.839 by this formula. There should, however, be an additional term ($p_{X\cdot O}r_{EO}$) in the formula to allow for correlation through other factors (O) than W, T, R, and H. From Table III we find that evaporation is determined

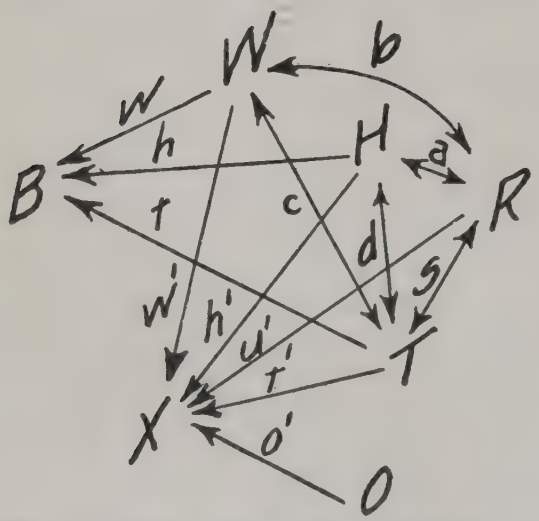


FIG. 16.—Relations between evaporations or transpiration (X) and the system shown in figure 15.

to a considerable extent ($d_{E\cdot O}=0.161$) by outstanding factors. The additional term in this case would have this value and when added to 0.839 gives 1, as it should. With one exception, the calculated correlation between transpiration and evaporation is a little smaller than the actual correlation. This means either that there is some additional factor which should be allowed for or else that the path coefficients with W, T, R, and H are not given quite their due weight, owing perhaps to lack of complete linearity in the correlations.

TABLE II.—*Table of calculated path coefficients*

	Wind.	Temperature.	Radiation.	Absolute humidity.	Correlation with evaporation.	
					Calculated.	Actual.
	$p_{X\cdot W}$	$p_{X\cdot T}$	$p_{X\cdot R}$	$p_{X\cdot H}$	r_{XE}	r_{XE}
Wet-bulb depression	0.298	0.896	0	−0.811	0.830	0.83
Evaporation (shallow tank)	.395	.544	.395	−.437	(.839)	1.00
Transpiration:						
Small grains	.238	.779	.249	−.489	.826	.87
Rye	.209	.853	.207	−.583	.852	.91
Sorghum and millet	.234	.718	.203	−.421	.741	.713
Sudan grass (inclosure)	.539	.870	.130	−.216	.838	.93
Sudan grass (open)	.339	.928	.059	−.375	.788	.82
Dent corn	.297	.815	.109	−.405	.751	.79
Algerian corn	.349	.851	.194	−.391	.844	.85
Cowpea and lupine	.351	.710	.214	−.346	.768	.775
Alfalfa	.303	.603	.117	−.424	.645	.705
Amaranthus	.052	.560	.105	−.428	.518	.560
Average transpiration	.279	.733	.181	−.420	.751	.781

TABLE III.—*Coefficients of determination*

	Wind.	Temperature.	Radiation.	Absolute humidity.	Joint determination.					Residual.
	$d_{X\cdot W}$	$d_{X\cdot T}$	$d_{X\cdot R}$	$d_{X\cdot H}$	$d_{X\cdot WT}$	$d_{X\cdot WR}$	$d_{X\cdot TR}$	$d_{X\cdot TH}$	$d_{X\cdot RH}$	$d_{X\cdot O}$
Wet-bulb depression	0.089	0.803	0	0.657	−0.011	0	0	−0.538	0	0
Evaporation	.156	.296	0.156	.191	−.009	−.003	0.202	−.176	+0.026	0.161
Transpiration:										
Small grain	.057	.607	.062	.240	−.007	−.001	.182	−.283	+.019	.125
Rye	.044	.728	.043	.340	−.007	−.001	.166	−.369	+.018	.038
Sorghum and millet	.055	.516	.041	.177	−.007	−.001	.137	−.224	+.013	.293
Sudan (inclosure)	.290	.757	.017	.047	−.019	−.001	.106	−.140	+.004	(−.062)
Sudan (open)	.115	.861	.003	.141	−.013	−.000	.051	−.258	+.003	.096
Dent corn	.088	.664	.012	.164	−.010	−.001	.084	−.244	+.007	.237
Algerian corn	.122	.724	.038	.153	−.012	−.001	.155	−.247	+.012	.057
Cowpea and lupine	.123	.504	.046	.120	−.010	−.002	.143	−.182	+.011	.247
Alfalfa	.092	.364	.014	.180	−.007	−.001	.067	−.190	+.008	.474
Amaranthus	.003	.314	.011	.183	−.001	−.000	.055	−.178	+.007	.607
Average transpiration	.078	.537	.033	.176	−.008	−.001	.124	−.228	+.012	.277

The coefficients of determination are given in Table III. The difference between their sum and unity is given in the last column as $d_{X\cdot O}$, the determination by outstanding factors. As suggested above, the assumption that all the fundamental correlations are linear may involve

some error which would tend to underweight the coefficients of determination between transpiration and the known factors and so overweight the apparent degree of determination by outstanding factors. In certain cases, however, the residue is so small, in one case actually coming out negative, that it is probable that this is not an important source of error. The residual determination is greatest for the crops which were cut twice during the season—namely alfalfa and amaranthus. There were considerable periods following each cutting during which the absolute value of the transpiration was small.

Wind velocity has about the same relative value as a factor in determining transpiration as it has in determining wet-bulb depression. Its relative importance is a little greater for determining evaporation from the shallow tank.

Temperature is somewhat more important than absolute humidity in determining the variations in wet-bulb depression and rate of evaporation from day to day. It is very much the most important factor in determining the rate of transpiration in all the plants.

Radiation is an important factor in evaporation, coming out equal to wind velocity and only slightly less important than absolute humidity. In the plants, on the other hand, it is almost a negligible factor.

Comparing transpiration in the average plant with evaporation in the sun from a shallow tank, we find that the former is influenced relatively much more by temperature, to about the same degree by absolute humidity, somewhat less by wind velocity, and very much less by radiation. The four factors are much more nearly equal in importance in the case of evaporation ($d_{E\cdot T}=0.30$, $d_{E\cdot H}=0.19$, $d_{E\cdot W}=0.16$, $d_{E\cdot R}=0.16$) than in the case of transpiration ($d_{X\cdot T}=0.55$, $d_{X\cdot H}=0.18$, $d_{X\cdot W}=0.09$, $d_{X\cdot R}=0.04$). In comparing the importance of these factors it should be added that radiation has an importance somewhat in excess of its direct influence, in that its variations are correlated with those of temperature. Humidity has reduced importance, since, though correlated with temperature, it affects evaporation and transpiration in the opposite direction.

OTHER APPLICATIONS

The method of analysis presented here can readily be applied to the problem of the relative importance of heredity and environment. An application of this kind to the case of the piebald pattern of guinea pigs has already been published (9), and one to the resistance of the same animal to tuberculosis is in press.[1] The method can be applied also to such a problem as the determination of the effects of various systems of mating, such as inbreeding, line breeding, and assortative mating on the genetic composition of an originally random-bred stock.[2]

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MEASUREMENT OF THE AMOUNT OF WATER THAT SEEDS CAUSE TO BECOME UNFREE AND THEIR WATER-SOLUBLE MATERIAL

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INTRODUCTION

It has been shown that soils cause water to become inactive or unfree, as is indicated by its refusal to freeze or to function as a solvent. The magnitude of this unfree water has been measured by means of the dilatometer method,[1] which has proved most convenient, appropriate, and unique for this purpose. The principle of this method is based upon the fact that water expands upon freezing. If the amount of expansion that a certain amount of water (1 gm.) produces upon freezing is known, then the quantity of water that freezes in the soil can be calculated from the magnitude of expansion produced. On the basis of this dilatometer method the water in the soil has been classified anew as follows:

1. Gravitational water, unsuitable to plants.
2. Free water, readily available to plants.
3. Unfree water { Capillary, adsorbed, very slightly available to plants. / Combined { water of hydration / water of solid solution } very unavailable to plants.

The free water is that which freezes very readily at the supercooling of $-1.5°$ C.; the capillary, adsorbed water is that which freezes from the temperature of $-1.5°$ to $-78°$; while the combined water is that which does not freeze at all, even at the extreme temperature of $-78°$.

AMOUNT OF WATER THAT SEEDS CAUSE TO BECOME UNFREE

It is, of course, very well known that seeds absorb large quantities of water and with a considerable force. Seeds like the lima bean, cowpea, soybean, clover, and alfalfa absorb over 100 per cent of their dry weight of water; while seeds like the wheat, rye, and corn absorb about 50 per cent of their dry weight of water. The great attraction that seeds have for water is best realized by the fact that they will abstract the moisture from the soils even down to the point of air-dryness. Whitney

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and Cameron [1] found, for instance, that when 50 gm. of seeds of cowpeas were mixed with 50 gm. of soil containing 15 per cent of water, the seeds had in 12 hours gained 12.1 per cent of water and had left in the soil only 1.3 per cent—that is, the soil was reduced practically to air-dry condition. It appears, therefore, that the power of seeds to absorb water is very much greater than that of soils. Some attempts have been made to measure the magnitude of the initial attraction that seeds possess for water. Especially notable is the work in this direction of Shull [2] who attempted to measure the attraction of seeds of Xanthium for water, and then he used these seeds to measure in turn the moisture-holding forces of soils. Shull found that the air-dry seeds of Xanthium show an initial attraction for water of nearly 1,000 atmospheres.

Since it was found that soils cause water to become unfree, the extent varying with the character of the soil, the question arose whether the seeds also cause water to become unfree, and if so, to what extent. It was reasoned and anticipated that since seeds possess a greater attraction for water as evidenced by their power to abstract moisture from the soil itself even down to the point of dryness, they ought to cause a larger amount of water to become unfree.

In order to obtain information bearing upon these questions a general investigation of the problem was undertaken. The type of dilatometer used and the general procedure followed were the same as those used in the study of soils. The procedure consisted in weighing out carefully about 10 gm. of air-dry seeds and placing them in water to soak for about two days. Then they were taken out, pressed between filter papers in order to eliminate their excess of water, weighed again quickly, and introduced into the dilatometer. The unoccupied space in the dilatometer was then filled with ligroin, and care was taken to expel all the air. The mouth of the dilatometer was then carefully stoppered, and the contents were placed to cool in a temperature of $-3°$ C. When this temperature was attained by the contents, as indicated by the column of ligroin in the stem, which remained stationary, the water in the seeds was caused to freeze. This was accomplished by taking hold of the dilatometer by the stem and moving it gently in the cooling mixture until solidification began, which was indicated by the rise of the ligroin in the stem. The dilatometer was allowed to remain in the cooling mixture with frequent movements until the rise of the ligroin in the stem ceased. The total rise of the ligroin in the stem was taken to represent the total amount of expansion due to the formation of ice.

In order to determine the effect of repeated freezing or of lower temperature upon the amount of water that seeds cause to become unfree, the

[1] WHITNEY, Milton, and CAMERON, F. K. INVESTIGATIONS IN SOIL FERTILITY. U. S. Dept. Agr. Bur. Soils Bul. 23, p. 30. 1904.

[2] SHULL, Charles Albert. MEASUREMENT OF THE SURFACE FORCES IN SOILS. *In* Bot. Gaz. v. 62, no. 1, p. 1–31, 5 fig. 1916. Literature cited, p. 29–31.

seeds in the dilatometer were thawed and refrozen either at the temperature of $-3°$ or of $-20°$ C. In the latter case, the contents of the dilatometer were allowed first to supercool at $-3°$ and to assume equilibrium at this temperature; then they were put in the temperature of $-20°$, allowed to remain there for about one hour, and were then placed back into the temperature of $-3°$ and allowed to attain equilibrium.

In all, 14 different kinds of seeds were used. These were spring wheat, winter wheat, barley, rye, white corn, yellow corn, broom corn, alfalfa, alsike clover, mammoth clover, cowpeas, field peas, field white beans, and black soybeans.

In Table I are presented part of the data obtained. They show the amount of water the different kinds of seeds absorbed and the quantity they caused to become unfree, as indicated by its refusal to freeze for the first time at the temperature of $-3°$ C. The quantity of unfree water is expressed both in cubic centimeters and in percentage based on the weight of the air-dry seeds. The factor used for converting the volume of expansion due to the ice formation into the corresponding weight of water was that obtained experimentally and used in the study of the soil—namely, 1 cc. of water expands approximately 0.1 cc. upon freezing.

TABLE I.—*Amount of water that failed to freeze in seeds when they were supercooled and frozen for the first time in a temperature of $-3°$ C.*

Kind of seeds.	Weight of air-dry seeds.	Weight of water-soaked seeds.	Absorbed water which failed to freeze.	
	Gm.	*Gm.*	*Cc.*	*Per cent.*
Spring wheat	11.210	18.290	2.880	25.70
Winter wheat	11.250	18.510	3.360	30.10
Barley	10.770	18.080	4.310	40.02
Rye	10.230	18.150	3.920	40.20
White corn	12.075	17.640	3.765	31.18
Yellow corn	12.215	17.265	4.650	38.09
Brown corn	10.020	16.230	2.510	25.05
Alfalfa	11.300	25.730	8.430	74.60
Alsike clover	11.200	24.200	7.400	66.08
Mammoth clover	11.200	25.700	7.100	63.40
Cowpeas	9.210	20.790	6.680	72.54
Field peas	10.070	20.800	7.730	76.76
Field white peas	10.275	20.320	5.445	52.96
Black soybeans	7.110	16.920	5.310	74.68

From the foregoing experimental data it is at once seen that the amount of water which the seeds cause to become unfree is really very great in nearly all the different kinds of seeds. It varies from about 25.05 per cent with broom corn to 76.76 per cent with black soybeans. It appears that the alfalfa, clover, cowpeas, and bean seeds cause a considerably larger amount of water to become unfree than the wheat, rye, barley, and corn seeds. As has already been mentioned, this

percentage of unfree water is based only on the absorbed water; the hygroscopic moisture is not included in it. Hence the total amount of unfree water in the seeds is still greater than is represented by these numerical data.

In the foregoing investigation the seeds were supercooled and frozen only once in the temperature of −3° C. The investigations with soils revealed the fact that repeated freezing and thawing and lower temperature tended to reduce the amount of unfree water in soils, especially in the fine-textured and colloidal soils. In order to ascertain whether repeated freezing and thawing and lower temperature brought also a diminution in the unfree water in the seeds, the latter were frozen and thawed three times in a temperature of −20°. Finally they were supercooled to −3°, frozen in −20° for one hour, and brought back again to−3°, where the total expansion was measured. Table II contains the results obtained from this investigation. For immediate and convenient comparison the results obtained at the first freezing are also presented in this table.

TABLE II.—*Effect of repeated freezing and thawing and low temperature on the amount of water that fails to freeze in seeds*

Kind of seeds.	Water which failed to freeze, (frozen only once).	Water which failed to freeze, (frozen and thawed four times).	Difference in favor of seeds frozen only once.
	Per cent.	*Per cent.*	*Per cent.*
Spring wheat	25. 70	25. 70	0. 00
Winter wheat	30. 10	28. 98	1. 12
Barley	40. 02	35. 38	4. 64
Rye	40. 20	35. 39	4. 81
White corn	31. 18	23. 70	7. 48
Yellow corn	38. 09	26. 62	11. 47
Broom corn	25. 05	13. 08	11. 97
Alfalfa	74. 60	40. 98	33. 62
Alsike clover	66. 08	40. 18	25. 90
Mammoth clover	63. 40	41. 08	22. 32
Cowpeas	72. 54	39. 95	32. 59
Field peas	76. 76	57. 90	18. 86
Field white peas	52. 96	26. 78	26. 33
Black soybeans	74. 68	47. 96	26. 72

It is readily seen that repeated freezing and thawing has a very marked diminishing effect on the unfree water in the seeds, especially with certain kinds of seeds. In those seeds which contained a tremendous amount of unfree water at the first freezing, such as the alfalfa, clover, peas, and beans, the diminution in the quantity of unfree water by repeated freezing and thawing is very considerable, amounting in some cases to over 33 per cent. On the other hand, in such seeds as the

wheat, corn, barley, and rye the process of repeated freezing and thawing had very little effect if any on the unfree water.

The process of repeated freezing and thawing, therefore, has practically the same influence in seeds as it has in soils. In both cases it tends to diminish the amount of unfree water in some seeds or soils more than in others.

In explaining the decrease of the unfree water by repeated freezing and thawing two hypotheses were presented. In the one it was suggested that part of the water is held by the capillarities of the soil and does not freeze. Upon repeated freezing and thawing these capillarities are destroyed, and the water they held is liberated or becomes free and freezes readily.

In the second hypothesis it was assumed that soils such as clays, clay loams, silts, muck, and peats contained a considerable amount of colloidal material which held water in such a manner that it does not freeze. Upon repeated freezing and thawing, however, these colloids are coagulated or destroyed, and the water they held is liberated or becomes free and readily freezes.

These suggested explanations with few modifications may apply also to seeds. There is no doubt that the living tissue as well as its capillaries and colloidal material are affected or destroyed by severe freezing.

It may be of interest to record here that when very old corn seed was employed or corn seed that had been frozen in the field, no water was caused to become unfree. Apparently long age or previous freezing of the corn seed destroyed its power to cause water to become unfree. This phenomenon, however, did not appear in the other seeds.

According to the classification of moisture in the soils based on the dilatometer method, the water which freezes after the first freezing may be classified as capillary-adsorbed water, while that which refuses to freeze after the fourth freezing and at the low temperature may be classified as combined, probably in the form of water of hydration and water of solid solution.

However, the division of the unfree water into capillary adsorbed and combined water is probably not so sharp in seeds as in soils, because in the seeds there is a considerable quantity of water-soluble material which causes a high freezing-point depression, and this in turn decreases the amount of water that freezes at the degree of supercooling employed. As is well known, there is always a tendency for an equilibrium to be established between the liquid-solvent, solid-solvent, and the solute at any temperature below freezing until the cryohydric temperature is reached. Some of the water, therefore, which refused to freeze at -20° C. or which froze and melted again at -3° may be due to the water-soluble material of the seeds. It is believed, however, that the amount of water that was prevented from freezing by the high freezing-point depression of the seeds is probably not very great.

AMOUNT OF WATER-SOLUBLE MATERIAL IN SEEDS AS MEASURED BY THE FREEZING-POINT METHOD

Recognizing the influence that high concentration of solution has upon the quantity of water that refuses to freeze, the authors always determined the freezing-point depression [1] of the seeds after they were used for the dilatometer measurements. It was found that the magnitude of this depression was high for most of the seeds. Since the seeds, however, used in the dilatometer measurements were allowed to stand about two days in excess of water and were then subjected to alternate freezing and thawing, it was thought that the depression values obtained were the result of the biological and physical changes that the seeds underwent. In order to ascertain, however, whether the seeds contained water-soluble material in the dry condition they were ground very fine and then portions of 10 gm. were mixed with 20 cc. of water in a freezing-point tube. The mixture was allowed to stand for about 40 minutes, and then its freezing-point depression was determined in the usual way. Table III contains the results obtained. The values of the freezing-point depression have also been calculated into osmotic pressure in atmospheres after the table of osmotic pressures worked out by Harris and Gortner.[2]

TABLE III.—*Freezing-point depression and osmotic pressure of dry seeds when 10 gm. of powdered dry seeds were mixed with 20 cc. of water*

Kind of seeds.	Freezing-point depression.	Osmotic pressure.
	° *C.*	*Atmospheres.*
Spring wheat	0.280	3.375
Rye	.352	4.243
Buckwheat	.280	3.375
White corn	.340	4.098
Broom corn	.280	3.375
Sorghum	.580	6.988
Alfalfa	.610	7.349
Alsike clover	.650	7.830
Mammoth clover	.650	7.830
Cowpeas	.715	8.612
Field peas	.550	6.628
Field white beans	.685	8.251
Black soybeans	.560	6.747
Speckled wax beans	1.180	13.336
Red kidney beans	1.060	12.760

The results in Table III are very surprising. They show most strikingly that there is a tremendous amount of readily water-soluble material in seeds, and in some seeds much more than in others. Thus the depression varies from 0.280° C. in wheat to 1.180° in speckled wax beans. When

[1] BOUYOUCOS, George J., and MCCOOL, M. M. OP. CIT.

[2] HARRIS, J. Arthur, and GORTNER, Ross Aiken. NOTES ON THE CALCULATION OF THE OSMOTIC PRESSURE OF EXPRESSED VEGETABLE SAPS FROM THE DEPRESSION OF THE FREEZING POINT, WITH A TABLE FOR THE VALUES OF P FOR Δ=0.001° TO Δ=2.999°. *In* Amer. Jour. Bot., v. 1, no. 2, p. 75–78. 1914.

it is considered that this relatively large depression is obtained in a ratio of 1 of seeds to 2 of water (10 gm. of seeds and 20 cc. of water), then it can be imagined what the depression must be at a very low moisture content. It really must be large. In the ratio given here it varies from 3.375 atmospheres in wheat to 13.336 in speckled wax beans. The great attraction that seeds possess for water and their ability to abstract it from soils even down to the point of air-dryness must be due, therefore, partly, if not largely, to their great osmotic pressure caused by their high content of easily water-soluble material.

No experimental work was performed to prove definitely the nature of the material in the seeds which went into solution to cause such great depression. But it appears to be largely water-soluble proteins such as albumins and probably also some of the mineral bases. It can not be starch, which is the most abundant constituent in the seeds, because that is very insoluble in water. A test showed, for instance, that 10 gm. of starch in the pure form in 20 cc. of water had a depression of only 0.025° C. Sugar, of course, which is soluble, is not supposed to be found in dry seeds. Furthermore, to give the high depression obtained, there has to be present a very large amount of sugar, because as it is well known that this class of material does not dissociate. All evidences, therefore, point to the proteins as the main class of constituents in the seeds which produced such high depressions in the freezing point when dry seeds in the powdered form were mixed with water.

SUMMARY

Seeds cause part of the water which they absorb to become unfree, as is indicated by its refusal to freeze.

The dilatometer method is a convenient and appropriate method for measuring the magnitude of this unfree water in seeds.

The amount of water that seeds cause to become unfree is very large, varying from 25.05 per cent in broom corn to 76.76 per cent in black soybeans, based on the air-dry weight of seeds. Repeated freezing and thawing tends to diminish considerably the amount of unfree water, especially in some seeds.

Dry seeds contain a large amount of water-soluble material, as is evidenced by the high freezing-point depression. When 10-gm. portions of seed flour are mixed with 20 cc. of water and the mixture is allowed to stand for about 40 minutes or less, the freezing-point depression varies from 0.280° C. in wheat to 1.180° in speckled wax beans. At very low moisture content the magnitude of this depression must be very great. The magnitude of the osmotic pressure must also be correspondingly very great.

The great power that seeds possess to absorb water and to abstract it from the soil is partly if not largely due to their tremendous internal osmotic pressure.

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INHERITANCE OF SYNDACTYLISM, BLACK, AND DILUTION IN SWINE[1]

By J. A. DETLEFSEN, *Professor and Chief in Genetics*, and W. J. CARMICHAEL,[2] *Associate in Animal Husbandry, Illinois Agricultural Experiment Station*

Our present point of view in animal and plant breeding is being shaped to a large extent by experiments in the field of genetics. Probably the plant breeders have profited more by these experiments than the animal breeders; for there are relatively few precise observations on inheritance in domestic mammals, for obvious reasons. While the animal breeder can not afford to neglect the conclusions obtained with pedigreed cultures of laboratory material, nevertheless the data accumulated directly from domestic mammals will more quickly stimulate clear thinking and intelligent practice. For these reasons, among others, the following observations are presented and put on record.

The data in this study are derived from an original cross between a single pure-bred registered mule-foot boar and a number of pure-bred Duroc-Jersey sows, eligible to registration. Both boar and sows were owned by Mr. J. H. Percival, of Champaign, Ill. The results of the cross were so striking and uniform that we were invited to examine the progeny born in the fall litters of 1915 and in the spring litters of 1916. All the F_1 offspring, about 250 in number,[3] were self-colored black and mule-footed. Furthermore, the progeny resembled the mule-foot boar in general conformation (in which, as a matter of fact, both the sire and the Duroc-Jersey sows were much alike). The case is a good illustration of one type of prepotency, where the sire is homozygous in a number of conspicuous dominant characters, such as black and mule-foot in this particular instance. But the progeny inherited as much from their dams as they did from the sire, as the next generation showed. The vigorous hybrids were raised for the market and not for further breeding purposes, as is the case generally with such hybrids. Since the material seemed

[1] Paper No. 9 from the Laboratory of Genetics, Illinois Agricultural Experiment Station.

[2] Resigned May 31, 1918, to become Extension Animal Husbandman, United States Department of Agriculture; at present Secretary of the National Swine Growers' Association. The writers are indebted to Mr. J. B. Rice, Associate in Animal Husbandry, for much assistance after the resignation of the junior writer.

[3] The number of F_1 young in this paper is conservatively estimated at about 250. The exact number can not be given because the animals were kept in a large pasture, which made an exact count difficult. There is no doubt, however, that all F_1 individuals were black and mule-footed.

promising for further genetic investigation, six F_1 sows were purchased in June, 1916.

The six sows, numbered 1 to 6 in Tables I and II, were bred back to a Duroc-Jersey boar, since this type was recessive in a number of characters in the original cross. Each female, except ♀ 5, gave at least one litter, and ♀ 3 gave two litters. A total of 42 F_2 offspring by this backcross was thus obtained. The original mule-foot P_1 parent was without doubt homozygous in mule-foot and black and probably had the genetic formula BBMM, where B is a factor for black and M is a factor for mule-foot. The Duroc-Jerseys had the genetic formula bbmm, where b stands for red, and m for cloven-foot. The F_1 hybrids were then heterozygous in both black and mule-foot (BbMm) and, if the case is one of simple Mendelism, produced gametes BM + Bm + bM + bm with equal frequency. Mating the F_1 females to the Duroc-Jersey male should give in Mendelian terms:

BM +	Bm +	bM +	bm	F_1 gametes
bm +	bm			Duroc-Jersey gametes
BbMm +	Bbmm +	bbMm +	bbmm	F_2 zygotes
black	black	red	red	
mule-foot	cloven	mule-foot	cloven	

That is, the F_2 classes would be of four equally frequent types. The calculated and observed results agree, for there were produced 8 black mule-foot, 11 black cloven-foot, 9 red mule-foot, and 14 red cloven-foot where 10.5 of each kind is the calculated result. (See Pl. 70.) So far as the evidence goes, the allelomorphic pair of factors for syndactylism and cloven-foot is quite independent of the allelomorphic pair for black and red. The ultimate recessive segregates, red cloven, bred true when mated *inter se* and gave 30 red cloven in the F_3 and F_4 generations.

TABLE I.—*Distribution of F_2 segregates from mule-foot × Duroc-Jersey F_1 hybrids mated back to Duroc-Jersey*

Dam No.	Offspring.								Total.
	Males.				Females.				
	Black mule-foot.	Black cloven-foot.	Red mule-foot.	Red cloven-foot.	Black mule-foot.	Black cloven-foot.	Red mule-foot.	Red cloven-foot.	
1	.....	.....	1	.....	.....	.....	3	2	6
2	.....	4	1	.....	.....	.....	.....	3	8
3	1	2	.....	.....	.....	2	.....	.....	5
3	1	.....	.....	3	.....	1	1	1	7
4	.....	1	1	1	2	1	1	1	8
6	2	.....	.....	3	2	.....	1	.....	8
Total	4	7	3	7	4	4	6	7	42

TABLE II.—*Original data on the F_2, F_3, and F_4 offspring from a cross of a mule-foot boar on Duroc-Jersey sows*

F_1 Dam No.	Duroc-Jersey sire.	Off-spring No.	Sex.	Color.	Foot character.	Date of birth.	Remarks.
1	Good Colonel, No. 41517	1 a	♀	Red	Cloven	Nov. 8, 1916	
		1 b	♀	Yellow	do		
		1 c	♂	Cream	Mule		
		1 d	♀	do	do		
		1 e	♀	Lemon	do		
		1 f	♀	Yellow	do		
2	do	2 a	♂	Black	Cloven	Mar. 7, 1917	Saved for breeding.
		2 b	♂	do	do		
		2 c	♂	do	do		
		2 d	♂	do	do		
		2 e	♀	Yellow	do		Do.
		2 f	♀	Lemon	do		
		2 g	♀	Red	do		
		2 h	♂	Yellow	Mule		
3	do	3 a	♀	Black	Cloven	Nov. 8, 1916	
		3 b	♀	do	do		
		3 c	♂	do	do		
		3 d	♂	do	do		
		3 e	♂	do	Mule		
3	do	3 f	♂	Yellow	Cloven	Mar. 13, 1917	Saved for breeding.
		3 g	♂	Black	Mule		
		3 h	♂	Yellow	Cloven		
		3 i	♂	do	do		
		3 j	♀	Black	do		
		3 k	♀	Cream	Mule		Some roan.
		3 l	♀	Red	Cloven		
4	do	4 a	♀	Black	do	Jan. 19, 1917	
		4 b	♀	do	Mule		
		4 c	♀	do	do		
		4 d	♀	Yellow	do		
		4 e	♀	Cream	Cloven		Some roan.
		4 f	♂	do	Mule		
		4 g	♂	Red	Cloven		
		4 h	♂	Black	do		
6	do	6 a	♂	Yellow	do	Apr. 8, 1917	Slightly roan.
		6 b	♂	Cream	do		
		6 c	♂	Red	do		Much roan.
		6 d	♂	Black	Mule		
		6 e	♂	do	do		
		6 f	♀	Red	do		
		6 g	♀	Black	do		
		6 h	♀	do	do		White spot on upper lip.

F_2 dam No.	F_2 sire No.	Off-spring No.	Sex.	Color.	Foot character.	Date of birth.	Remarks.
2 e	3 f	2e–a	♂	Red	Cloven	Mar. 1, 1918	Some doubt as to degree of red in this litter.
		2e–b	♂	do	do		
		2e–c	♂	do	do		
		2e–d	♂	do	do		
		2e–e	♀	do	do		

TABLE II.—*Original data on the F_2, F_3, and F_4 offspring from a cross of a mule-foot boar on Duroc-Jersey sows*—Continued.

F_2 dam No.	F_2 sire No.	Offspring No.	Sex.	Color.	Foot character.	Date of birth.	Remarks.
2 f.....	3 f.....	2f–a	♂	Cream.	..do....	Mar. 5, 1918	Saved for breeding.
		2f–b	♀	..do....	..do....		Do.
		2f–c	♀	..do....	..do....		Do.
		2f–d	♀	..do....	..do....		
		2f–e	♀	..do....	..do....		
		2f–f	♂	Yellow.	..do....		
		2f–g	♂	..do....	..do....		
		2f–h	♂	..do....	..do....		
		2f–i	♂	..do....	..do....		
		2f–j	♀	..do....	..do....		
		2f–k	♀	..do....	..do....		
		2f–l	♀	..do....	..do....		
		2f–m	♀	..do....	..do....		

F_3 dam No.	F_3 sire No.	Offspring No.	Sex.	Color.	Foot character.	Date of birth.	Remarks.
2f-b....	2f-a.....	2f–b–a	♂	Cream.	Cloven.	Mar. 18, 1919	Lemon on top of head and shoulders.
		2f–b–b	♀	..do....	..do....		
		2f–b–c	♀	..do....	..do....		
		2f–b–d	♀	..do....	..do....		
2f-c....	2f-a.....	2f–c–a	♂	Cream.	..do....	Apr. 16, 1919	
		2f–c–b	♀	..do....	..do....		Few yellow hairs between ears.
		2f–c–c	♀	..do....	..do....		
		2f–c–d	♂	Yellow.	..do....		
		2f–c–e	♂	Red....	..do....		
		2f–c–f	♂	Yellow.	..do....		
		2f–c–g	♀	..do....	..do....		
		2f–c–h	♀	Cream.	..do....		

Syndactylism has been recognized as an inherited character in man by Lewis and Embleton (*6*),[1] Lewis (*5*), and Pearson (*7*); in poultry by Davenport (*3*); and in swine by Spillman (*9*). In man there is probably one main dominant factor allelomorphic to normal; and the case shows simple Mendelism as we now understand it, although both Lewis and Embleton and Pearson were not inclined to such a view. In poultry, Davenport concluded that syndactylism was very imperfectly dominant to its allelomorph, normal toes. Syndactylism versus cloven-foot in swine has been cited as an illustration of monohybridism in a number of textbooks, but no published data are available. Spillman states:

> It is interesting to note that in crosses between mule-foot hogs and ordinary breeds the mule-foot character seems to be dominant.

[1] Reference is made by number (italic) to "Literature cited," p. 604.

No statement is made regarding segregation. Kronacher (*4*) implied that the character was transmitted pure after hybridization, for he says:

> Der Züchter v. Dunin-Kozicky liess im Jahre 1888 ein derartiges, gelegentlich erhaltenes Einhuferschwein (polnisches Landschwein) von einem Yorkshireeber decken und erhielt zur Hälfte (5 von 9) solche Einhufernachzucht, die ihr charakteristisches Merkmal rein Eitervererbte.

It is difficult to know whether Kronacher really means that these mule-foot hybrids gave no cloven-footed segregates or that the character when transmitted showed no contamination after the cross and was therefore "pure." Reference to the original source quoted by Kronacher leaves no doubt as to segregation, for von Dabrowa-Szremowicz (*1*) states explicitly that, in attempting to fix the mule-foot character, sporadic cases of cloven-foot crop out. He says:

> Da bei den Schweinen es überhaupt schwer ist, eine einheitliche und gleichmässige Abart festzustellen, so treffen sich auch noch bei den meinigen vereinzelte Fälle mit gespaltenen Hufen.

It is clear, then, that this case agrees with both Spillman's and our own observations on dominance and with our observations on segregation.

The original mule-foot boar in these crosses was undoubtedly homozygous (MM) in the factor for syndactylism, for every one of his offspring, about 250, showed the mule-foot character. Six F_1 sows (Mm) were bred back to the cloven-foot Duroc-Jersey (mm), and each one gave both mule-foot and cloven-foot segregates. The total F_2 generation thus produced was 17 mule-foot + 25 cloven-foot, where theory calls for 21 of each kind as the most probable result. The deviation, 4, is no larger than one might reasonably expect as a fluctuation of sampling $\left(\frac{\text{deviation}}{\text{probable error}} = \frac{4}{2.19} = 1.83\right)$. If we add to these results those recorded by von Dabrowa-Szremowicz, we obtain 22 mule-foot + 29 cloven, where 25.5 is the most probable value. In this total, the calculated and observed results show even a closer agreement, for $\frac{\text{deviation}}{\text{probable error}} = \frac{3.5}{2.41} = 1.45$.

In experiments with the larger domestic mammals the usual apology for small numbers must be made, for they often obscure the real facts. In making our results a test against a monohybrid Mendelian hypothesis we must not overlook the fact that our data might also admit of a dihybrid interpretation with interaction of two factors to produce the mule-foot character. For example, if mule-foot were due to the interaction of X and Y, then the original mule-footed grandparent was XXYY and, mated to xxyy females, gave XxYy in the F_1 generation. Back-crossing to xxyy would thus be supposed to give:

$$\underbrace{\text{XxYy}}_{\text{25 per cent mule-foot}} + \underbrace{\text{Xxyy} + \text{xxYy} + \text{xxyy}}_{\text{75 per cent cloven-foot}}.$$

We observed a ratio of 17 mule-foot to 25 cloven-foot in the F_2 generation, while on this latter hypothesis the calculated results would be 10.5 to 31.5. The $\frac{\text{deviation}}{\text{error}} = \frac{6.5}{1.89} = 3.43$. The odds against deviations as wide or wider are about 45 to 1. But if we again add the results of von Dabrowa-Szremowicz to ours, the observed ratio is 22 to 29, where 12.75 to 38.25 is the calculated ratio. In these combined results the $\frac{\text{deviation}}{\text{error}} = \frac{9.25}{2.09} = 4.43$. The odds against deviations as wide or wider are now about 350 to 1. In both cases the monohybrid explanation is much more satisfactory. Furthermore, on a dihybrid hypothesis we should sometimes obtain mule-footed when F_2 cloven-footed segregates are mated together. To test this, such matings were made. Two of the three cloven-foot F_2 daughters of ♀ 2 (♀ 2e and ♀ 2f in Table II) were bred to a cloven-foot F_2 son of ♀ 3 (♂ 3f, Table II).[1] One F_2 ♀ gave 5 F_3 cloven-foot (4 ♂ ♂ + 1 ♀) and the other F_2 ♀ gave 13 F_3 cloven-foot (5 ♂ ♂ + 8 ♀ ♀). Therefore, a total of 18 F_3 cloven-foot was obtained from F_2 cloven-foot segregates bred *inter se*. In the F_3 generation two cloven-foot ♀ ♀ (♀ 2f-b and ♀ 2f-c) were mated to their cloven-foot brother, ♂ 2f-a, and gave 4 and 8 cloven-foot respectively. We may conclude that mule-foot and cloven-foot represent a single allelomorphic pair, in which the syndactylous form is dominant and the normal form is recessive, and that extracted recessives breed true.

As is common among mule-foot swine, the fused phalanges may separate along the line of fusion as the animal becomes older and heavier. This splitting was infrequent in the front feet, but was occasionally seen in the hind feet. There was never any difficulty in classifying the syndactylous and normal at the time of birth or when the animals were young, for syndactylism is a distinct discontinuous variation from normal. There is, however, some variation in syndactylism itself. Fusion may vary from complete, with no trace of separation on the hoof, to a less perfect fusion with two deep parallel lines of demarcation. The former condition is characteristic of the front feet, while the latter is the usual condition in the hind feet. In an examination of 17 F_2 mule-foot segregates, 14 showed complete fusion in the front feet, but 16 showed the deep lines of demarcation on the hind feet. The factor for syndactylism acts differently on the front and hind feet. (See Pl. 70.)

The relation of black to red in swine has never been quite clear. It is well known that Poland China or Berkshire mated to Duroc-Jersey usually produces a tortoise-shell type of red and black, but the amount of each color varies markedly. Sandy, yellow, cream, or even white may be substituted for red in such crosses, as shown by Severson (8). Wright (10) advanced a suggestive hypothesis that such a tortoise-shell type of sandy colored hog with black spots was selected in two directions to give the characteristic color of the Berkshire or Poland China, on the one

[1] The relationship of all animals recorded in this paper may be obtained from Table II, the original data.

hand, and Duroc-Jersey or Tamworth on the other. Selecting on the basis of minor factors for the extension of black and for the dilution of red to white gave the Berkshire color type, while selecting minor factors for the restriction of black and for the intensity of red gave the Duroc-Jersey type. In our crosses the self-black of the mule-foot does not act like the black of the Berkshire with its peculiar pattern, but whether this is due to a real difference in their genetic factors for black or is due to variable spotting factors in the Berkshire as compared with the self of the mule-foot remains to be shown. The six white points of the Berkshire may represent a highly selected spotting factor, or factors, with numerous modifiers. By crossing such Berkshires to Duroc-Jerseys one would expect to obtain a complex spotted hybrid. The mule-foot and the Duroc-Jersey are both self-colored and, as our experiments indicate, a cross between the two involves no striking spotting factors but shows clear-cut segregation between self-red and self-black. We may, therefore, regard black (B) as a dominant allelomorph to red (b) in our crosses.

The original mule-foot boar (BB) was mated to Duroc-Jerseys (bb) and gave about 250 F_1 hybrids (Bb) which were self-black. The 6 F_1 sows mated to a Duroc-Jersey boar gave 19 blacks to 23 reds in the F_2 generation, where 21 of each kind is the calculated ratio. The recessive F_2 red segregates gave 18 F_3 reds. The F_3 reds when mated *inter se* gave 12 F_4 reds. Extracted recessive reds, therefore, breed true. The total results indicate that black and red are allelomorphs in this cross in swine, black being dominant to red. In all of the foregoing discussion the term red includes red, yellow, lemon, and cream shades—that is, any form showing red pigment but no black.

In any wide cross between two distinct varieties like the Duroc-Jersey and mule-foot there are many factorial differences involved, and we are not surprised to find numerous new variations in the F_2 generation and subsequent hybrids which were not seen in the parents. Thus, we observed an occasional white spot on the feet or hoofs, white spot on the upper lip, animals with varying amounts of roan, more variation in size, and the like. Among the more striking variations seen in the F_2 generation were the grades of intensity and dilution of red pigment. Although red in the Duroc-Jersey varies somewhat, the red F_2 segregates varied much more than the original Duroc-Jersey parents. The black of the F_1 and F_2 hybrids, on the contrary, did not vary perceptibly. This seems to indicate that diluters of red may be carried by black swine but that such diluters do not affect black. In this cross the original black mule-foot sire evidently contributed diluters of red to the black F_1 hybrids; and such diluters segregated, giving more variability in red in the F_2 generation. We can hardly suppose that the Duroc-Jerseys contributed the factors for this dilution, because Duroc-Jerseys mated *inter se* do not show such dilute forms as we observed among our red segregates. Samples of hair from each individual were saved. Comparing all F_2 reds with each other, we classified these around four more or less arbitrary

modes—red, yellow, lemon, and cream, given in the order of most intense to most dilute. Those classified as "cream" were, when adults, a very light straw color, almost white. The 23 F_2 individuals were distributed as follows: 6 red, 9 yellow, 2 lemon, and 6 cream. It is not certain that yellow and lemon belong to two genetically distinct classes. There is little difference between them. If we group yellow and lemon together as an intermediate shade, the ratio of 6 intense, 11 intermediates, and 6 dilutes suggests a 1:2:1 ratio; but this is probably a coincidence, and we can not infer a single allelomorphic pair of factors for intensity and dilution with incomplete dominance, as later experiments will show. We do not know what the calculated ratio for the various shades of red in such an F_2 population should be, for we do not know the genetic constitution of each F_1 female or the Duroc-Jersey male with regard to these diluters of red. This one fact, however, is clear—there was marked segregation in shades of red in the F_2 generation. Plate 70 shows some of the variation in the intensity of red.

In order to test these dilute conditions, ♀ 2e, an F_2 yellow, and ♀ 2f, an F_2 lemon, were mated to ♂ 3f, an F_2 yellow. It was thought that if yellow and lemon were intermediate conditions between cream and red, then these matings would give a range of forms from red to cream. Female 2e gave 5 F_3 offspring classified as red. They were discarded, and unfortunately some doubt exists as to the exact shade of red. The shade of red deepens as the animals grow older. We are quite sure they were not cream, but they may have been either yellow or red. Some were a deeper, more intense color than either F_2 parent. Female 2f gave 13 F_3 young, of which 5 were cream and 8 were yellow. The creams when born were absolutely white, and a microscopic examination of their hair cleared in xylol and mounted in Canada balsam showed no pigment. Later in life they acquired some yellow pigment in the medulla of the hair but little or none in the cortex and gave the general appearance of a very light straw-color. The presence of red, yellow, and cream among the F_3 offspring from yellow parents suggested that yellow might after all be an intermediate condition and that a lighter shade like cream is recessive. We did not know at that time whether a single pair of factors with incomplete dominance was involved or whether there were a number of independent factor pairs for yellow, the cumulative effect of which gave the more intense shades

If a single alleomorphic pair with incomplete dominance were responsible, then all the offspring from the F_3 creams should have been cream. Three F_3 animals (♂ 2f-a, ♀ 2f-b, ♀ 2f-e) classified as cream (white at birth but very light straw-color when adults) were bred *inter se* to give the F_4 generation. However, the offspring from these creams were not all cream, for ♀ 2f-b produced 4 creams, but ♀ 2f-c gave 4 creams, 3 yellows, and 1 red. This hypothesis, therefore, becomes untenable. The difference between the creams and yellows or reds in this last litter, as in all others, was a distinct one, and there can be no question as to the

accuracy of classification. The fact that yellow may give red, yellow, and cream and that the cream-colored may give red, yellow, and cream leads us to believe that there is an interaction of factors producing intensity of red and that similar somatic creams are not necessarily of the same genetic constitution. The case appears much like the belt in the Hampshire, where either belted × belted or nonbelted × nonbelted may give both forms; or like purple and white aleurone in maize, where either white × white or purple × purple may give both forms. We may add that the adult creams can hardly be distinguished from Chester White or Yorkshire color. Under the microscope these creams show little or no pigment in the cortex of the hair but show yellow granules in the medulla. Some white hairs from the Berkshire and Chester White may also show yellow pigment. We have seen white hairs from the Berkshire which show yellow pigment in the medulla exactly like our creams.

The fact that red hair may be so diluted as to be almost if not quite indistinguishable from white hair suggests that the so-called white hair in some breeds may really be a very dilute red. Severson's experiments (*8*) show that Berkshire mated to Duroc-Jersey may give white and black spotted rather than the usual red or yellow and black. If the white hair of the Berkshire is really a dilute red, such a result would be expected in occasional matings of Berkshire to Duroc-Jerseys carrying recessive diluters; and there seems to be much evidence that Duroc-Jerseys carry recessive dilution factors, for much lighter animals than the standards require are known. Severson mated such a white and black hybrid back to a Berkshire and obtained some red and black offspring. Disregarding the black, this mating is like our matings of two creams which gave reds, and it thus adds weight to our hypothesis that the intenser shades, like red and yellow, are due to interaction of at least two pairs of independent factors; but the more dilute shades, like cream or white, are due to the absence of one or both interacting factors. That is, zygotes with both interacting factors A and B would be red or yellow, while zygotes with either A or B, or neither, would be cream or white. The fact that creams or whites form one distinct grade and yellow and red form another leads us to believe that the two groups are quite distinct. The slight variations in red and yellow or in the creams may be due to other minor factors. Summarizing, we may say that there are three sources of evidence which indicate that cream or white may be dilute red, that dilution and intensity are complex characters due to interaction of independent factors, and that the so-called white hair in some breeds is really a cream or very dilute red, as follows: (1) Yellow pigment was found in the medulla of the hair of our creams and in the white hair of Berkshires, (2) red offspring were derived from our creams mated *inter se*, and (3) red and black spotted offspring were derived from Severson's white and black spotted hybrid (from Duroc Jersey × Berkshire) mated to Berkshire.

SUMMARY

Syndactylism in swine is allelomorphic and dominant to normal cloven-foot, and black is allelomorphic and dominant to red. The two pairs of factors are evidently independent of each other.

The factor for syndactylism does not show quite the same effect on the front feet as on the hind feet, for the fusion is usually less complete in the latter.

The Duroc-Jersey and mule-foot are both self-colored in this cross and transmit no distinct spotting factors. We have concluded tentatively that the hybrids between Duroc-Jersey and Berkshire (or Poland China) are spotted because the latter transmit highly selected dominant spotting factors.

Intensity of red appears to be due to the interaction of independent factors which do not affect black. Dilution of red or yellow to cream or white takes place when either one or neither of the interacting factors is present. The so-called white hair of some breeds like the Berkshire and Poland China is really a very dilute red of genetic composition similar to our cream segregates.

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PLATE 70

The four types of F_2 segregates from a cross between mule-foot boar and Duroc-Jersey sows.

A.—Black mule-foot.

B.—Black cloven foot.

C.—Red mule-foot.

D.—Red cloven foot.

There is much variation in the intensity of red. The fusion in the hind feet is less pronounced than in the front feet.


Front Feet
Hind Feet
A
B
C
D

FOUR RHYNCHOPHORA ATTACKING CORN IN STORAGE

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INTRODUCTION

Of the numerous insect enemies of stored corn there are four belonging to the suborder Rhynchophora, or weevils, that are to a greater or less extent of economic importance in the United States. Of these four, one has received but little attention from economic entomologists, while of the remaining three much has been published, but comparatively little careful work has been done with the immature stages.

It is the purpose of this paper to present accurate drawings of the immature stages of these weevils, together with carefully prepared descriptions and keys, so that the various species may be readily distinguished in whatever stage they may be found.

The weevils under discussion represent two different families, Anthribidae and Curculionidae, and three different genera, Araecerus, Caulophilus, and Sitophilus, two of the weevils belonging to the last genus.

KEY TO ADULTS

a. Beak short and broad.

b. Robust beetle, antennæ inserted in small foveæ upon the upper surface of base of beak, last three segments of antennæ forming a loose club. *Araecerus fasciculatus* DeG.

bb. Slender, elongate beetle, antennæ inserted at middle of beak, last few joints forming a compact club..... *Caulophilus latinasus* Say.

aa. Beak elongate and slender.

c. Thorax with coarse, sparse, elongate punctures, wings lacking. *Sitophilus granarius* L.

cc. Thorax with coarse, deep, very dense punctures, wings present. *Sitophilus oryza* L.

KEY TO MATURE LARVÆ

a. Body slender, elongate, supplied with some or many long hairs, abdominal segments with hypopleurum not subdivided, mandibles armed dorsally with a pair of bristles set close together.

b. Larger, 4.5 to 6 mm. in length, body profusely covered with long hairs. *Araecerus fasciculatus* DeG.

bb. Smaller, 2 to 2.5 mm. in length, body sparsely provided with long hairs..... *Caulophilus latinasus* Say.

[1] The writer wishes to express his gratitude to Dr. Adam G. Böving, of the Bureau of Entomology, United States Department of Agriculture, for his kindness in extending much valuable aid and advice in the study of the larval forms and the preparation of the technical descriptions.

aa. Body short and stout, armed with but few small setæ, abdominal segments with hypopleurum subdivided into three lobes, mandibles armed dorsally with a pair of bristles set far apart.

c. First three abdominal segments only, above divided into three distinct areas, middle lobe of hypopleurum without seta....*Sitophilus oryza* L.

cc. First four abdominal segments above divided into three distinct areas, middle lobe of hypopleurum armed with a seta. *Sitophilus granarius* L.

KEY TO PUPAL STAGES

a. Antennæ not geniculate, folded over on dorsum.....*Araecerus fasciculatus* DeG.

aa. Antennæ geniculate.

b. Beak short and broad.....*Caulophilus latinasus* Say.

bb. Beak elongate and slender.

c. Inner wings rudimentary, almost completely concealed by elytra.....*Sitophilus granarius* L.

cc. Inner wings well developed, extending well beyond tips of elytra.....*Sitophilus oryza* L.

ARAECERUS FASCICULATUS [1]

SYNONYMY [2]

Araecerus fasciculatus DeG.

"DeGeer. *Ins. V, 1775. p. 276. t. 16. f. 2.*—Wollast. *Ann. Nat. Hist. V. 1870. p. 18.*—Lucas. *Ann. Fr. 1861. p. 399.*

cacao Fabr. *Syst. Ent. p. 64.*—Oliv. *Ent. IV. 80. p. 15. t. 2. f. 21. a–b.*

capillicornis Say. *Journ. Ac. Phil. V. 2. 1827. p. 249.*

moestus Lec. *Ann. Lyc. I. p. 172.*

cassiae Winthem. Dej. *Cat. 3. ed. p. 259.*

coffeae Fabr. *Syst. El. II. p. 411.*—Gylh. *Schh. Gen. Curc. I. p. 175*—Labr. et Imh. *Gen. Curc. I. nr. 55.*

crassicornis Fabr. *Ent. Syst. Suppl. p. 159; Syst. El. II. p. 399.*

griseus Steph. *Ill. Brit. IV. p. 211. t. 21. f. 2. (forte.)*

japonicus Thunb. *Nov. Act. Ups. VII, p. 122.*

perigrinus Herbst, *Käf. VII. p. 168. t. 106. f. 9.*

saltatorius Falderm. *in litt.*

var. *sambucinus* Boisd. *Voy. Astrol. II. p. 299 (forte.)*—MacLeay, *Dej. Cat. 3. ed. p. 259.*"

Araecerus fasciculatus (Pl. 71) was described in 1775 by DeGeer from Surinam. It is thought to have originated in India, but now it is cosmopolitan in distribution. This beetle, commonly known as the coffee-bean weevil, is robust, dark brown, and clothed with mottled light and dark brown pubescence. The beak is short and wide.

ADULT

Ovate, convex. Dark brown to black or piceous, clothed with yellowish and dark brown pubescence; intervals of elytra alternately tessellate with brown and yellowish; antennæ, tibiæ, and tarsi reddish brown, club fuscous; femora piceous at middle. Thorax very finely and exceedingly densely punctate. Elytra with rows of fine, close-set, feebly impressed punctures; intervals very finely and densely granulate-punctate. Length 2.5 to 4.5 mm.[3]

[1] Family Anthribidae, tribe Araecerini.

[2] GEMMINGER, M., and HAROLD, B. DE. CATALOGUS COLEOPTERORUM. v. 9, p. 2749. Monachii, 1872.

[3] BLATCHLEY, W. S., and LENG, C. W. RHYNCHOPHORA OR WEEVILS OF NORTH EASTERN AMERICA. p. 42. Indianapolis, Ind. 1916.

EGG

Egg shining, white, ovoid in shape; top broadly rounded, bottom slightly more pointed. Length about 0.56 mm., width 0.35 mm.

LARVA

Mature larva 4.5 to 6 mm. in length; white, footless, fleshy grub with body curved, wrinkled, and profusely covered with long hairs. Head very pale straw color; anterior margin and mandibles slightly darker. Head longer than broad, somewhat oblong in shape. Epicranial and frontal sutures faint and slightly lighter in color; there are also two longitudinal, light stripes rising from the frontal sutures and running to base of head. Frons almost triangular in shape; frons and epicranial lobes provided with numerous long hairs. Antenna small, situated at anterior corner of frons. Mandibles large, stout, triangular, with apex produced into an acute tooth; inner edge toward apex provided with two acute subapical teeth and above protractor with a large molas process or structure. Dorsal area of each mandible armed with a pair of stout bristle set close together. Eye represented by a well-defined black spot beneath the exoskeleton. Clypeus and labrum present, both broader than long and about equal in breadth. Labrum provided with four pairs of dorsal hairs and five pairs of short, thickened, marginal setæ; ventral surface of labrum with four small setæ. Maxillæ elongate, terminated by a 2-jointed palpus and a single setose lobe. Maxilla armed with numerous long hairs and a stout chitinized seta on palpifer just below the maxillary lobe. Stipes labii fused with the basal joint of the 2-jointed palps and bearing two setæ on each side. Ligula and lingua fused and marked by a seta on each side. Linguar region with numerous small asperities and a few setæ. Behind linguar region is a strong hypopharyngeal chitinization connected on each side with epicranium with well-developed hypopharyngeal bracon. Chitinization anteriorly provided with a cavity the bottom of which bears pointed processes. Posterior part of hypopharyngeal chitinization less heavily chitinized and limited by a chitinized frame which gradually continues over into floor of oesophagus. Mentum and submentum separated, mentum bearing two long hairs and submentum nine pairs of long hairs arranged in four groups of four each and a median pair.

Pronotum simple and not divided. Mesothoracic and metathoracic segments are above divided into three areas, representing praescutum, fused scuto-scutellar area, and postscutellar area; below and adjacent to epipleurum is alar area. Below ventrolateral suture are hypopleurum, coxal lobe, and eusternum, all well-defined and profusely provided with long hairs. Mesothoracic spiracle located on preepipleural lobe of mesothorax near prothorax; larger than abdominal spiracles and differing from them by being bifore whereas abdominal spiracles are monofore. Kidney-shaped air tubes pointing dorsad. Ten abdominal segments: Ninth, small; tenth, reduced; one to eight, each provided with monofore spiracles, that of eighth segment being located slightly more dorsad and with air tube pointing cephalad instead of dorsad. Praescutal and scutal areas of abdominal segments large and protuberant; scutal area, however, attenuating dorsad and not reaching the dorsal outline, scutellum and postscutellum flatter. Praescutum, scutum, and scutellum profusely armed with long hairs. Epipleural lobes bulging and prominent, also well supplied with long hairs.

Measurements of larval stages

STAGE.	WIDTH OF LARVAL HEAD.
1	0.22 mm.
2	.34 mm.
3	.58 mm.
4	.78 mm.
5	.90 mm.

PUPA

Pupa white when first formed, cast larval skin clinging tightly to last abdominal segments. Length 3.75 to 4 mm.; width 2 mm. Tips of elytra pointed and terminated with a long, chitinized hook nearly reaching seventh abdominal segment. Metathoracic tarsi extending well beyond tips of elytra. Head rounded, beak short and broad. Head profusely supplied with hairs. Antennæ nongeniculate, folded over on dorsum, tips nearly meeting on metanotum. Prothorax profusely supplied with long hairs, femora apically armed with several hairs. Mesonotum and metanotum each provided with two bunches or tufts of long hairs. Elytra armed with numerous hairs. Each abdominal segment is armed with two rows of dorsal, and numerous lateral, hairs. Seventh and eighth abdominal tergites apparently fused together; the ninth segment bears two large bilobed fleshy processes armed with numerous papillæ. The tenth segment is ventral to the ninth.

CAULOPHILUS LATINASUS [1]

SYNONYMY [2]

Caulophilus latinasus Say.

"*Rhyncholus latinasus*, Say, Descr. N. Am. Curc. p. 30 (1831) Complete Writings, 1. p. 299 (nec Boheman).

Caulophilus latinasus, Lec. Proc. Am. Phil. Soc. xv. p. 340 (1876); Champ. Ent. Monthly Mag. xlv. p. 121.

Caulophilus sculpturatus, Woll. Ins. Mader. p. 315, t. 6. figg. 4–4 *a–c* (1854).

Cossonus pinguis, Horn, Proc. Am. Phil. Soc. xiii. p. 442 (1873). Cossonus picipennis, Sturm, in litt."

Caulophilus latinasus (Pl. 72) was described from Florida in 1831 by Thomas Say. This weevil is now widespread over the State of Florida and has been reported from South Carolina and Georgia. It is also known to occur in Jamaica, Porto Rico, Mexico, Guatemala, and Madeira. It is doubtless common throughout the islands of the West Indies and in the countries of Central and South America.

It is commonly known as the "broad-nosed grain weevil," and is a slender, elongate, reddish brown weevil with a short, broad beak. Technical descriptions of the adult and immature stages follow.

ADULT

Elongate, rather robust. Reddish brown or piceous, feebly shining. Beak longer than half the thorax, sparsely punctured, with a faint elongate fovea between the eyes. Thorax as broad as long, moderately constricted near apex, sides strongly curved, base slightly narrowed, feebly bisinuate; disk rather finely and evenly punctured, with a broad, faint impression on basal third. Elytra subcylindrical, not wider than middle of and more than twice as long as thorax, moderately convex; striæ deep, rather coarsely and closely punctured on basal half, more finely or obsoletely near apex, the seventh and eighth united behind the humerus as in Allomimus; intervals convex, indistinctly punctulate. Under surface sparsely punctured. Front tibiæ sinuate within.

Length 3 mm.[3]

[1] Family Curculionidae, subfamily Cossoninae, tribe Cossonini.

[2] CHAMPION, G. C. RHYNCHOPHORA. CURCULIONIDAE. CURCULIONINAE (CONCLUDED) AND CALANDRINAE. *In* Biol. Centr.-Amer. INSECTA. COLEOPTERA. v. 4, pt. 7, p. 40. 1909–1910.

[3] BLATCHLEY, W. S., and LENG, C. W. OP. CIT., p. 535.

EGG

Egg opaque, shining white, bottom broadly rounded, top flattened and fitting into a translucent cap. Length, without cap, 0.45 to 0.47 mm.; width 0.27 to 0.32 mm.

LARVA

Mature larva 2 to 2.5 mm. in length, a white, footless, fleshy grub, with body curved and wrinkled. Head light brown or straw color, the anterior margin and mandibles a darker brown. Head about as broad as long, almost circular in form. Epicranial and frontal sutures distinct and light in color. There are also two oblique, longitudinal light stripes rising from the frontal sutures and coalescing with the epicranial suture near the base of the head. Frons subtriangular, with a distinct dark median line running from posterior angle to middle, and indicating carina. Frons provided with four pairs of large setæ, sutural margins each bearing one seta. Epicranial lobes each bearing the following setæ: One close to posterior angle of frons and located in the oblique, longitudinal stripe rising from the frontal suture, one small seta posterior to this and near occiput, two anterior to it on disk of epicranium, two opposite middle of frons, one opposite middle of mandible, one opposite hypostomal angle of mandible and one on hypostoma near base of mandible. Epistoma represented by thickened anterior margin of the front. Pleurostoma represented by somewhat darker, declivous area surrounding the mandibular foramen. Mandibles stout, triangular, with the apex produced into an acute apical tooth. Inner edge toward apex provided with a subapical tooth and a small medial tooth, no molar structure. Dorsal area of each mandible armed with a pair of stout bristles set close together. Eye represented by a well-defined black spot beneath exoskeleton. Clypeus broad at base, sides narrowing toward apical angles; distinctly broader but not as long as labrum. Epistomal margin provided with two fine hairs on each side. Labrum about as broad as long, rounded in front, provided with three pairs of large setæ and five pairs of short, thickened, marginal setæ.

Maxillæ terminated by a 2-jointed palpus and setose maxillary lobe. Maxillæ each provided with four setæ as follows: One on first segment of palpus, two on vaginant membrane between palpus and palpifer, and one stouter and larger one midway between palpus and cardo. The stipes labii enforced posteriorly by a median triangular chitinization bear 2-jointed palpi and a single pair of setæ. Ligula bearing four small setæ. Mentum and submentum fused and bearing three large setæ on each side.

Pronotum simple and undivided; praescutal and scuto-scutellar areas roughly indicated by rows of setæ. Mesothoracic and metathoracic segments divided above into two areas representing praescutum and scuto-scutellum; below and adjacent to epipleurum is the alar area. Below ventro-lateral suture are a well-defined hypopleurum, coxal lobe, and eusternum. The thoracic spiracle, located on the preepipleural lobe of mesothorax, is bifore, with the fingerlike air tubes pointing dorsad, and is somewhat larger than the abdominal spiracles. Ten abdominal segments; ninth small, tenth reduced. Each tergum of first eight abdominal segments divided above into three distinct areas, praescutum, scutum, and scutellum. Below and adjacent to epipleurum is the alar area. Abdominal segments provided with setæ as follows: Two on praescutum, five on scutellum, two on alar area, two on epipleurum, one on coxal lobe, and two on eusternum. Each of the first eight abdominal segments bears a bifore spiracle, that of the eighth being slightly larger than the rest.

Measurements of larval stages

STAGE.	WIDTH OF LARVAL HEAD.
1	0.22 to 0.23 mm.
2	.33 to .38 mm.
3	.53 to .57 mm.

PUPA

Pupa white when first formed. Length 2.8 to 3 mm.; width about 1.3 mm. Tips of elytra attaining the sixth abdominal segment, tips of metathoracic tarsi not extending beyond wing tips. Head rounded, beak short and broad. Head provided with two prominent spines towards vertex, two smaller ones on sides above eyes, a spine on each side of front between eyes, two pairs on beak between frontal ones and base of antenna, two pairs on beak between base of antenna and tip of beak, and four pairs of small setæ on tip of beak. Prothorax provided with two pairs of antero-marginal setigerous tubercules, one pair of antero-lateral, two pairs of postero-lateral, and four pairs of dorsal setigerous tubercules. Mesonotum and metanotum each provided with two pairs of spines. Abdomen with eight distinct dorsal tergites; dorsal area of each armed with two pairs of large spines; lateral area of each tergite armed with a spine at base of which is a small seta. Epipleural lobes each obscurely armed with one or two minute setæ. Ninth segment armed as usual with two prominent pleural spines.

SITOPHILUS ORYZA [1]

SYNONYMY [2]

Sitophilus oryza Linn. 1763.

"*oryzae* Linn. *Amoen. Ac. VI. 1763. p. 395.*—Oliv. *Ent. V. 83. p. 97. t. 7. f. 81. a–b.*—Gylh. *Schh. Gen. Curc. IV. p. 981.*—Scriba. *Stett. Zeit. 1857. p. 377.*—Kollar. *Sitzgsb. Wien. Ac. 1848. V. p. 3.*

frugilega Degeer. *Mem. V. p. 273.*

granaria Stroem. *Dansk. Vid. Selsk. Skrift., II. p. 56.*

quadriguttata Montrouz. *Ann. Fr. 1860. p. 910.*"

Var. *zea-mais* Motsch. Etudes Ent. IV, p. 77 (1855); Casey, Ann. N. Y. Acad. Sci. VI, p. 686.

Sitophilus oryza (Pl. 73) was described in 1763 by Linnaeus. It is thought to have originated in India, but it is now cosmopolitan in distribution. It is the predominant species of the grain weevils in the southern States of North America, where it is known as the "black or rice weevil." It is easily the commonest and most destructive grain weevil in the United States.

It closely resembles *Sitophilus granarius* in form but is readily distinguished by the presence of wings and the different punctuation of the thorax. Technical descriptions of the adult and immature stages follow.

ADULT

Reddish brown to piceous, opaque, elytra frequently with four rufous spots. Beak slender, cylindrical, three-fourths as long as thorax, at base slightly dilated, above with four rows of rather coarse punctures and with a slight fovea between the eyes. Thorax longer than wide, constricted near apex, sides feebly curved, gradually divergent to base; disc densely, deeply, and coarsely punctured. Elytra oblong, slightly narrowed at tip, deeply striate, striæ very coarsely and closely punctured; intervals slightly convex, narrow, the sutural with a row of coarse punctures; each puncture, both of thorax and elytra, bearing a very short yellowish seta. Beneath very densely and coarsely punctured.

Length 2.1 to 2.8 mm.[3]

[1] Family Curculionidae, subfamily Calandrinae.

[2] GEMMINGER, M., and HAROLD, B. DE. OP. CIT., v. 8, p. 2653. 1871.

[3] BLATCHLEY, W. S., and LENG, C. W. OP. CIT., p. 575.

EGG

Egg opaque, shining white, ovoid to pear-shaped in form, widest below middle, bottom broadly rounded, neck narrowing sharply toward top which is somewhat flat and bears a small rounded protuberance that fits into a cap or plug that cements the egg into place. Length 0.65 to 0.70 mm., width 0.28 to 0.29 mm.

LARVA

Mature larva 2.5 to 3 mm. in length, a pearly white, fleshy grub; very thick-bodied, ventral outline being approximately straight while dorsal outline is almost semicircular. Head light brown in color, anterior margin and mandibles much darker. Head longer than broad and somewhat wedge-shaped, sides broadly rounded from middle to apex, which is slightly angular. Sides nearly straight from middle to anterior angles, lateral area with an oblique, longitudinal, lighter stripe or area. Epicranial and frontal sutures distinct and light in color; also two oblique, longitudinal, light stripes rising from the frontal sutures and coalescing with the epicranial suture near base of head. Frons subtriangular with a distinct, dark median line indicating carina, running from posterior angle to beyond middle. Sutural margins irregular or sinuate. Frons provided with five pairs of large setæ, sutural margins each bearing a large seta. Each epicranial lobe with the following setæ: One close to posterior angle of frons and located within oblique longitudinal stripe rising from frontal suture, one very small seta posterior to this and near occiput, two anterior to it on disk of epicranium, two opposite middle of frons, one opposite middle of mandible, one opposite hypostomal angle of mandible, and one on hypostoma near base of mandible. Epistoma represented by thickened anterior margin of front, distinctly darker in color, with anterior margin declivous and slightly curving and lateral angles slightly produced and elevated where they support dorsal articulation of mandibles. Pleurostoma represented by darker declivous area surrounding mandibular foramen. Mandibles stout, triangular, with apex produced into a broad apical tooth; inner edge toward apex provided with a subapical tooth and a small medial tooth; no molar part. Dorsal area of mandible provided with a pair of stout bristles set apart. Eye represented by a well-defined black spot beneath exoskeleton.. Clypeus attached in front of frons and broadly transverse; broad at base, sides narrowing toward apical angles, slightly longer and broader than labrum, and bearing on epistomal margin two fine setæ on each side. Labrum distinctly broader than long with two small lateral and a larger rounded median lobe. Labrum provided with six large setæ behind middle, two marginal, short, thickened setæ on each of lateral lobes, and six similar marginal setæ on median lobe.

Maxilla with cardo present and distinct, stipes not divided into stipes proper, subgalea, and palpifer, but one continuous piece with the anterior inner angle produced into a single setose lobe. Palpus 2-jointed, bearing a single seta near apex of first segment. Three other setæ found on maxilla, two located on vaginant membrane between palpus and palpifer and one stouter and longer midway between palpus and cardo. No articulating maxillary area between maxilla and mental-submental region. Labium with submentum and mentum fused and represented by a broad lobe bearing three pairs of stout setæ. Stipes labii posteriorly enforced by a median, triangular chitinization, the anterior median section produced anteriorly between the palpi into a small lobe-like ligula which is fused with the lingua. Each stipes labii bears a single seta. Short, conical, 2-jointed palpi situated on anterior angles of stipites. Ligula bearing four small setæ. Prothorax not divided dorsally, but two areas, praescutal and scuto-scutellar, roughly indicated by rows of setæ. Mesothoracic and metathoracic segments divided above into two distinct areas, the anterior of which represents praescutum, and the posterior the scuto-scutellum and alar area. The thoracic spiracle is located on a lobe pushed into prothorax from epipleurum of mesothorax.

It is bifore, elongate, larger than abdominal spiracles and placed with the fingerlike air tubes pointing dorsad. Ten abdominal segments; ninth small, tenth reduced. Each tergum of first three abdominal segments divided above into three distinct areas, praescutum, scutum, and scutellum. Each tergum of fourth to eighth abdominal segments divided above into only two areas, first containing praescutal and scutal elements, second representing scutellum. Below these two areas and adjacent to the epipleurum is the alar area. Abdominal spiracles placed anteriorly and in a small separate corner piece, probably of alar area; spiracles bifore and found on abdominal segments one to eight, that on the eighth being located slightly more dorsad than the rest. Below a very indistinct and abrupt dorso-lateral suture and above a well-defined ventro-lateral suture is a large, not subdivided epipleurum. The abdominal epipleura are located considerably higher than the thoracic lobes. Below ventro-lateral suture is hypopleurum subdivided into three lobes, one directly under the other. Below hypopleurum is coxal lobe and below that sternum, consisting of eusternum and a posterior triangular area representing parasternum or parasternum fused with sternellum. Abdominal segments provided with setæ as follows: One on praescutum, a long and two short ones on scutellum, two on alar area located just above spiracle, two on epipleurum, one on coxal lobe, and two on eusternum. One of the setæ on the scutellum is usually missing on abdominal segments five to nine.

Measurements of larval stages

STAGE.	WIDTH OF LARVAL HEAD.
1	0.22 mm.
2	.32 mm.
3	.48 mm.
4	.64 mm.

PUPA

Pupa uniformly pearly white when first formed. Length 3.75 to 4 mm.; width about 1.75 mm. Tips of wing pads attaining seventh abdominal segment, tips of metathoracic tarsi extending beyond tips of inner wings. Head rounded, beak elongate and slender. Head with two prominent spines toward vertex, a group of two small spines and two spinules on each side above eyes, two pairs of small spines near anterior margin and one on each side of front between eyes. Three pairs of spines on beak between frontal ones and base of antenna, a pair of small ones on beak midway between base of antenna and tip of beak, a pair on sides of beak between latter pair and tip of beak, and two pairs of smaller ones on tip of beak. Prothorax provided with one pair of antero-marginal setigerous tubercules, one pair of antero-lateral, two pairs of medio-lateral, and four pairs of dorsal setigerous tubercules. Mesonotum and metanotum each provided with three pairs of spines. Abdomen has seven distinct dorsal tergites, the seventh being much larger than the rest, dorsal area of each armed with a pair of large and a pair of smaller spines. Lateral area of each tergite armed with a spine at base of which is a small seta. Epipleural lobes are each armed with two minute spines. Ninth segment is as usual armed with two prominent pleural spines.

SITOPHILUS GRANARIUS

SYNONYMY [1]

Sitophilus granarius Linn. 1758.

granarius "Linn. *Syst. Nat. Ed.* X. p. 378.—Panz. *Fn. Germ. 17. 11.*—Gylh. *Schh. Gen. Curc. IV. p. 977.*—Jacq. Duv. *Gen. Col. Curc. 1854. t. 29. f. 140.*—Frisch. *Beschr. All. Ins. 1720. II. p. 36. t. 8.*

pulicaria Panz. *ed. Voet. IV. p. 54. t. 37. f. 17.* (*forte.*)

segetis Linn. *l. c. p. 381.*

unicolor Marsh. *Ent. Brit. p. 275.*—Steph. *Ill. Brit. IV. p. 9.*"

Sitophilus granarius (Pl. 74) was described in 1758 by Linnaeus. It is thought to have originated in the regions of the Mediterranean, but is now widely distributed throughout the world. It occurs but seldom in the southern States of North America, preferring the cooler climate of the North.

It is a slender, cylindrical, chestnut-brown beetle with a slender, elongate beak. Technical descriptions of the adult and immature stages follow.

ADULT

Elongate-oblong, feebly convex. Chestnut brown to piceous, moderately shining. Beak two-thirds as long as thorax, slender, cylindrical, finely and sparsely punctate. Thorax sparsely punctate, punctures coarse and on the disc more or less fusiform. Elytra deeply striate, striæ punctured at bottom, not serrate; intervals smooth, alternately wider and more elevated, especially towards the base; the sutural with a row of elongate punctures. Pygidium coarsely cribate. Body beneath coarsely and less densely punctured than in *oryza*. Length 3 to 4 mm.[2]

EGG

Egg opaque, shining white, ovoid to pear-shaped in form, widest below middle, bottom broadly rounded, neck narrowing gradually toward top, which is somewhat flattened and bears a small rounded protuberance that fits into a cap or plug that cements the egg in place. Length 0.68 to 0.80 mm., width about 0.33 mm.

LARVA

Mature larva 2.5 to 2.75 mm. in length; a pearly white, footless grub, fleshy and very thick-bodied, ventral outline being approximately straight while dorsal outline is almost semicircular. Head and appendages of head similar in every respect to those of *Sitophilus oryza*. Thoracic segments similar in external appearance to those of *S. oryza*. The abdominal segments are similar in form to those of *S. oryza* with the following exceptions which afford the best characters for distinguishing between larvæ of these two species: First four abdominal segments divided above into three distinct areas, praescutum, scutum, and scutellum, whereas in the larva of *S. oryza* the first three only of the abdominal segments are so divided. Middle lobe of the hypopleurum of the abdominal segments of *S. granarius* is provided with a seta. This seta lacking in larva of *S. oryza*.

[1] GEMMINGER, M., and HAROLD, B. DE. OP. CIT., V 8, p. 2653. 1871.

[2] BLATCHLEY, W. S., and LENG, C. W. OP. CIT., p. 574.

Measurements of larval stages

STAGE.	WIDTH OF LARVAL HEAD.
1	0.25 to 0.26 mm.
2	.36 to .37 mm.
3	.47 to .48 mm.
4	.61 to .65 mm.

PUPA

Uniformly white when first formed; length 3.75 to 4.25 mm., width 1.75 mm. Tips of elytra attaining fifth abdominal segment, inner wings rudimentary and almost completely concealed by elytra. Tips of metathoracic tarsi extending beyond tips of elytra. Head rounded, beak elongate. Head has two prominent spines toward vertex, a group of two small spines and two spinules on each side above eyes, two pairs of small spines near anterior margin and one on each side of front between eyes, three pairs of spines on beak between frontal ones and base of antenna, a pair of small ones on beak midway between base of antenna and tip of beak, a pair on sides of beak between latter pair and tip of beak, and two pairs of minute spines on tip of beak. Prothorax provided with one pair of antero-marginal setigerous tubercules, one pair of antero-lateral, two pairs of medio-lateral, and four pairs of dorsal setigerous tubercules; also a pair of minute medio-lateral ventral spines. Mesonotum and metanotum normally each provided with three pairs of spines; one or more pairs often missing. Abdomen with seven distinct dorsal tergites, the seventh being much larger than rest. Dorsal area of each armed with a pair of large spines and a pair of smaller ones. Lateral area of each tergite armed with a spine, at base of which is a small seta. Epipleural lobes each obscurely armed with two minute setæ. Ninth segment armed as usual with two prominent pleural spines.

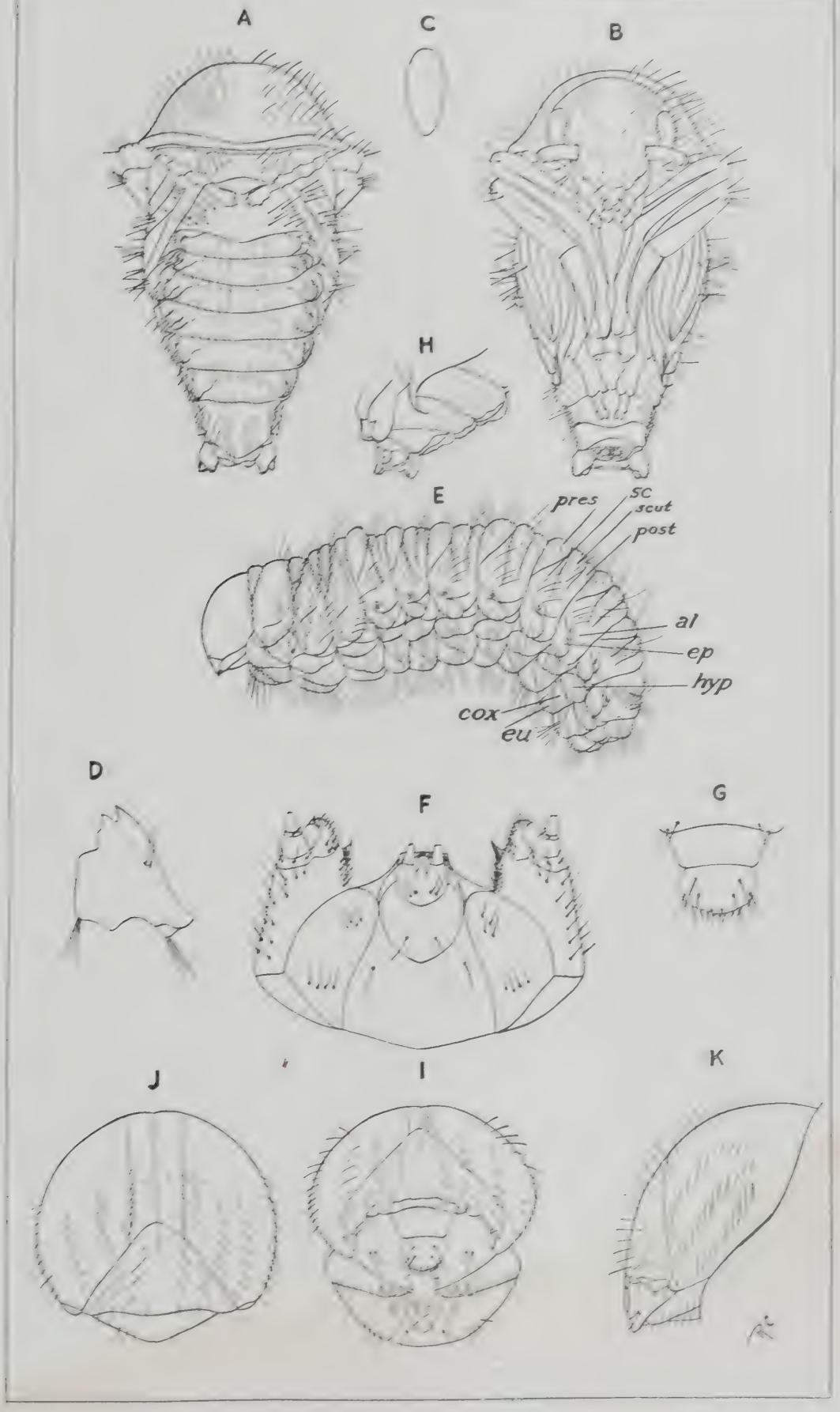
PLATE 71

Araecerus fasciculatus:

A.—Pupa, dorsal view.
B.—Pupa, front view.
C.—Egg.
D.—Mandible.
E.—Mature larva.
F.—Ventral view of head.
G.—Labium and clypeus.
H.—Pupa, lateral view.
I.—Head, face view.
J.—Head, dorsal view.
K.—Head, lateral view.

Key to larval parts

al=alar area.
cox=coxal lobe.
dlsut=dorso-lateral suture.
ep=epipleurum.
eu=eusternum.
hyp=hypopleurum.
par=parasternum.
post=postscutellum.
pres=praescutum.
sc=scutum.
scut=scutellum.
vlsut=ventro-lateral suture.


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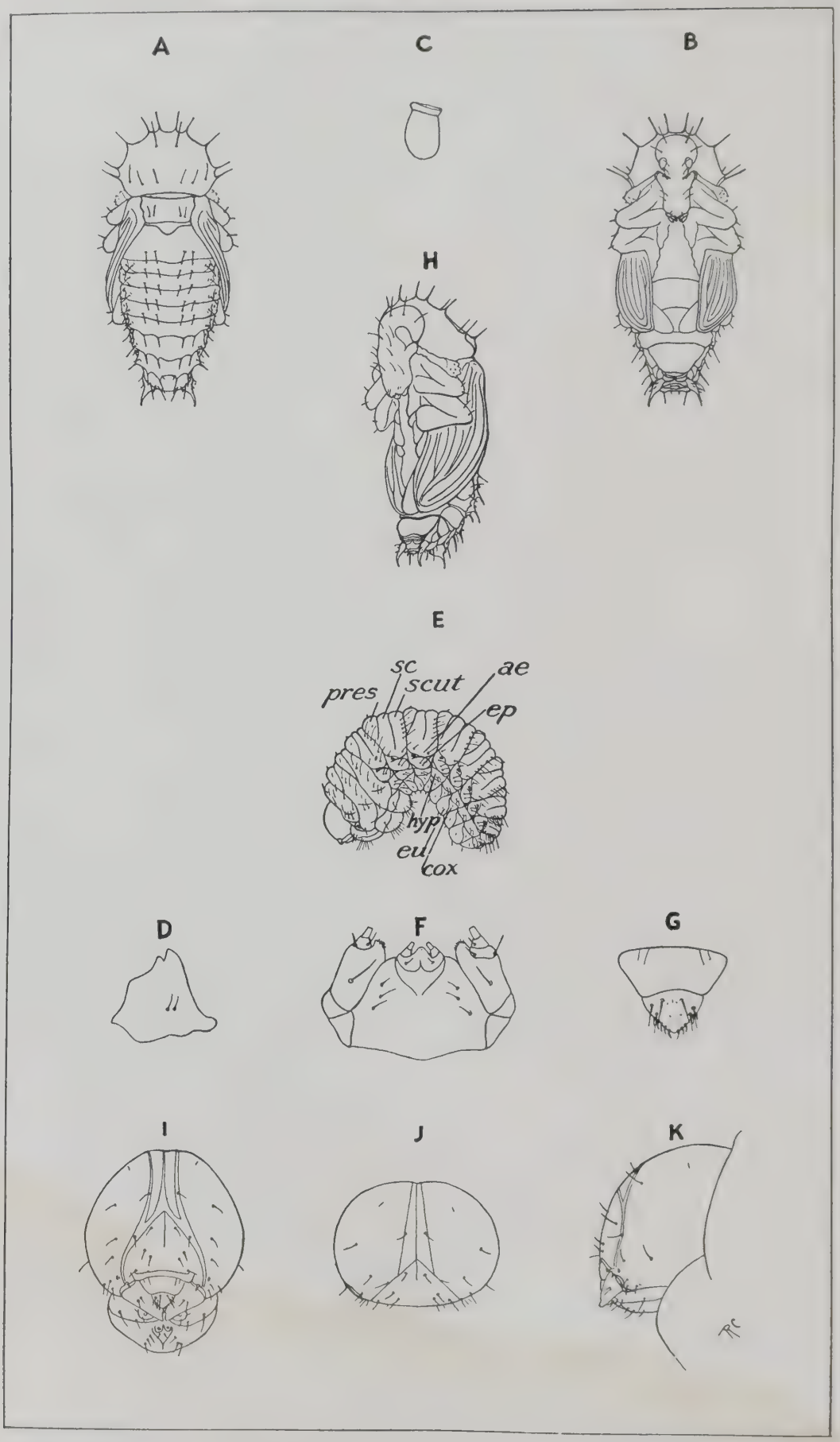

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PLATE 72

Caulophilus latinasus:

A.—Pupa, dorsal view.
B.—Pupa, front view.
C.—Egg.
D.—Mandible.
E.—Mature larva.
F.—Ventral view of head.
G.—Labium and clypeus.
H.—Pupa, lateral view.
I.—Head, face view.
J.—Head, dorsal view.
K.—Head, lateral view.

Key to larval parts

al=alar area.
cox=coxal lobe.
dlsut=dorso-lateral suture.
ep=epipleurum.
eu=eusternum.
hyp=hypopleurum.
par=parasternum.
post=postscutellum.
pres=praescutum.
sc=scutum.
scut=scutellum.
vlsut=ventro-lateral suture.

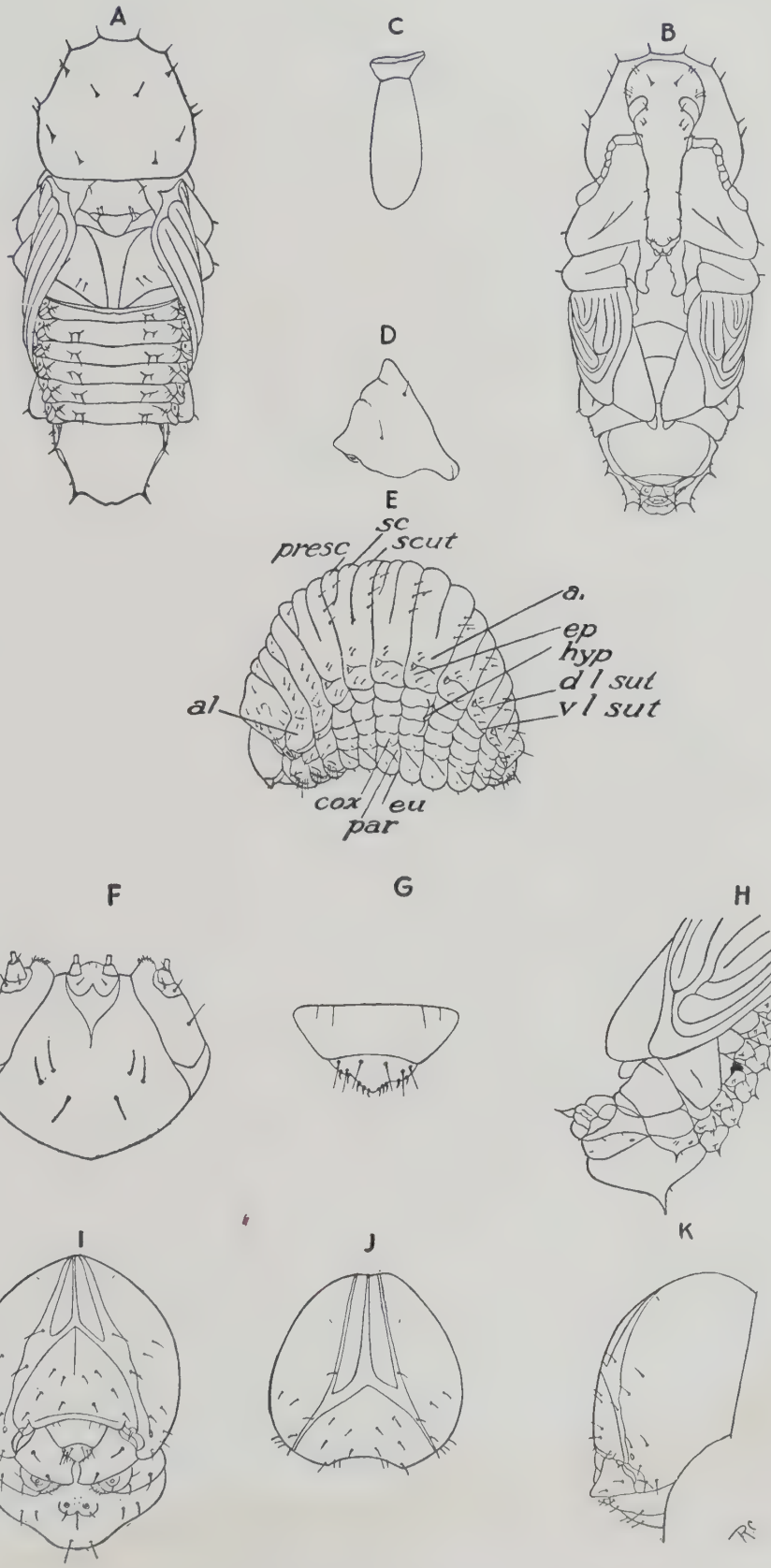
PLATE 73

Sitophilus oryza:

A.—Pupa, dorsal view.
B.—Pupa, front view.
C.—Egg.
D.—Mandible.
E.—Mature larva.
F.—Ventral view of head.
G.—Labium and clypeus.
H.—Pupa, lateral view.
I.—Head, face view.
J.—Head, dorsal view.
K.—Head, lateral view.

Key to larval parts

al=alar area.
cox=coxal lobe.
dlsut=dorso-lateral suture.
ep=epipleurum.
eu=eusternum.
hyp=hypopleurum.
par=parasternum.
post=postscutellum.
pres=praescutum.
sc=scutum.
scut=scutellum.
vlsut=ventro-lateral suture.


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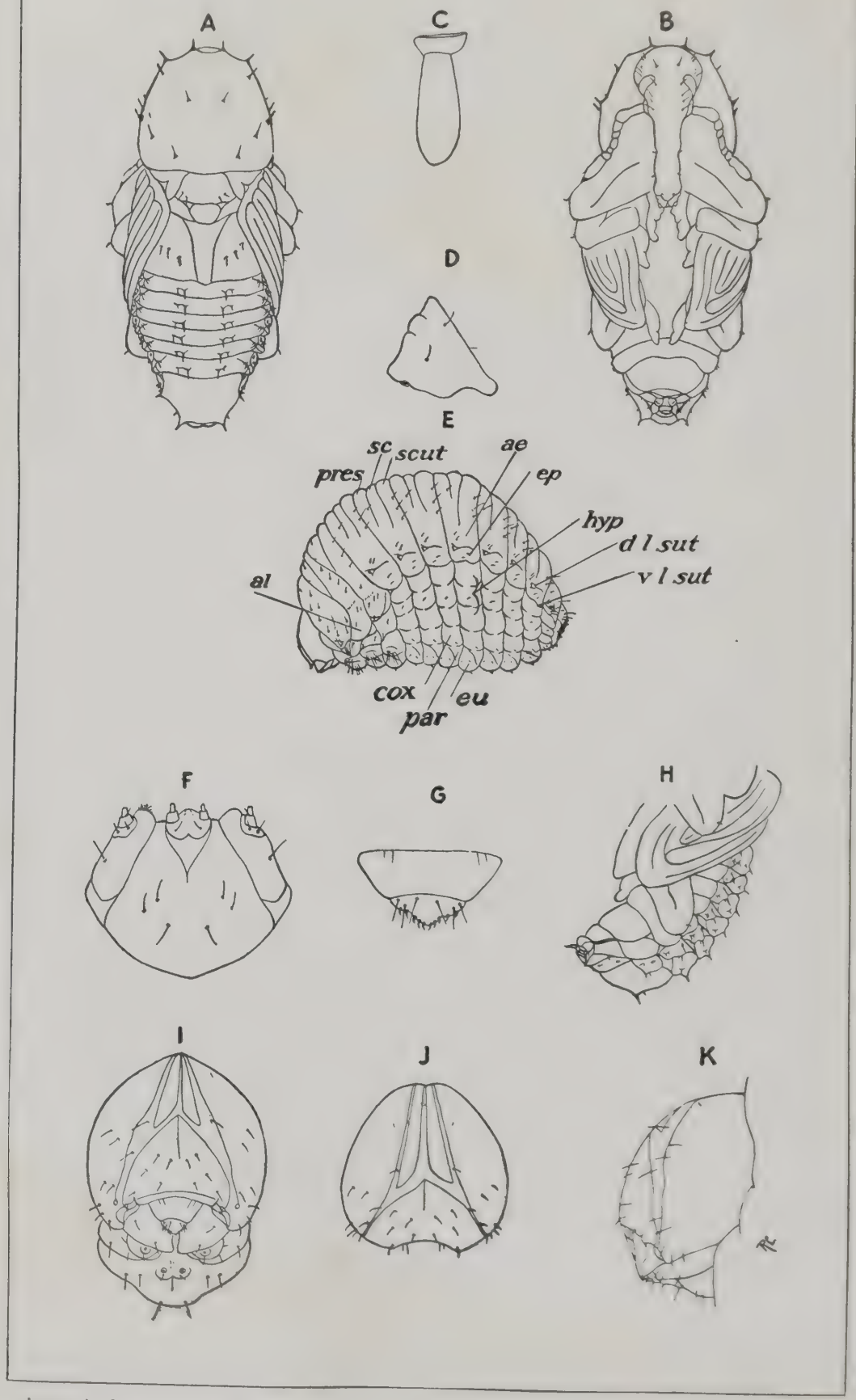

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PLATE 74

Sitophilus granarius:

A.—Pupa, dorsal view.
B.—Pupa, front view.
C.—Egg.
D.—Mandible.
E.—Mature larva.
F.—Ventral view of head.
G.—Labium and clypeus.
H.—Pupa, lateral view.
I.—Head, face view.
J.—Head, dorsal view.
K.—Head, lateral view.

Key to larval parts

al=alar area.
cox=coxal lobe.
dlsut=dorso-lateral suture.
ep=epipleurum.
eu=eusternum.
hyp=hypopleurum.
par=parasternum.
post=postscutellum.
pres=praescutum.
sc=scutum.
scut=scutellum.
vlsut=ventro-lateral suture.

CONCENTRATION OF POTASSIUM IN ORTHOCLASE SOLUTIONS NOT A MEASURE OF ITS AVAILABILITY TO WHEAT SEEDLINGS

By J. F. Breazeale, *Associate Biochemist*, and Lyman J. Briggs, *Physicist in Charge, Office of Biophysical Investigations, Bureau of Plant Industry, United States Department of Agriculture*

The object of the experiments described in this paper was to determine the availability of the potassium in solution of orthoclase by growing wheat seedlings in aqueous orthoclase solutions, analyzing the seedlings for potassium, and comparing the results with those obtained from suitable controls. The results show that the potassium present in solutions of orthoclase is not appreciably absorbed by young wheat plants. The conclusion is reached that potassium may be present in soil solutions in such combination with other elements that it is not available to plants.

The orthoclase used in our experiments was obtained near Riverside, Calif., and contained a total of 12.5 per cent of potassium oxid (K_2O). It was ground to pass a 60-mesh sieve. Different samples when brought into equilibrium with water and analyzed[1] contained from 2 to 9 parts per million of soluble potassium, the saturation concentration not being definite. There was, however, always some potassium present in the aqueous solutions, the average concentration being about 4 parts of potassium oxid per million of solvent.

The wheat was germinated on perforated aluminum disks floated on water. When the plumules were about ½ inch long the seedlings were transferred to other aluminum disks in the pans containing the culture solutions. This early transfer prevents the young seedling plants from absorbing the potash which exudes form unsprouted seeds.

The method of experimentation was, in general, to compare the potassium content of wheat seedlings grown in orthoclase solutions with that of similar seedlings grown in distilled water or other suitable control solution free from potassium.

SOLUBLE POTASSIUM IN ORTHOCLASE NOT AVAILABLE TO WHEAT SEEDLINGS

Wheat cultures were grown in orthoclase solution with and without the addition of gypsum and were compared with cultures grown in distilled water alone and in distilled water to which gypsum had been added. (See Table I, series a.) Although the orthoclase solutions were known

[1] The J. Lawrence Smith method was used in the analysis.

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from analyses to contain potassium, it will be noted that the wheat seedlings were unable to absorb any of it. This is of special interest, since the avidity of wheat seedlings for potassium is very marked.

The culture solutions in series b, Table I, included a control of distilled water (No. 1), 40 gm. of finely ground orthoclase in 2,500 cc. of distilled water (No. 3), and potassium chlorid solution containing 4 parts per million of potassium oxid (No. 5). Culture solutions Nos. 2, 4, and 6 were similar to No. 1, 3, and 5, respectively, except that gypsum was added to each in excess, so that it would always be present in the solid phase. To each of the six cultures were added also 50 parts per million of nitrate (NO_3) as sodium nitrate and 50 parts per million of phosphoric acid (P_2O_5) as sodium phosphate. Each solution, except those in which orthoclase was present in the solid phase, was changed twice daily in order to insure uniformity in concentration and freedom from bacterial disturbances. The wheat seedlings were grown in these culture solutions for 10 days. The analyses of the plants indicated, as before, that the wheat seedlings were unable to remove potassium from the orthoclase solutions. This was not due, however, to the diluteness of the solution, for in culture solutions containing only 4 parts per million of potash as potassium chlorid the plants were able to more than double their potash content in 10 days. The addition of nitrogen and phosphoric acid to the solutions did not modify the nonavailability of the potassium in the orthoclase solutions.

In series c the cultures were maintained for 17 days, all solutions being changed daily. Nitrogen and phosphoric acid were added to one culture, the sodium base being omitted. The results again showed no marked absorption of potassium from the orthoclase solutions.

The plants in series d were grown for 15 days. The analyses, as in the preceding experiments, showed no appreciable absorption of potassium by plants grown in orthoclase solutions, but a marked absorption was observed by plants grown in solutions of potassium chlorid. The presence of gypsum or orthoclase in the potassium chlorid solutions did not modify the rate of absorption of potassium from these solutions by the wheat seedlings.

The results in Table I, taken as a whole, show that the potassium in orthoclase solutions is not absorbed in measurable quantity by the wheat seedlings. On the other hand, potassium in potassium-chlorid solutions of equivalent concentration is readily absorbed by the plants.

TABLE I.—*Relative availability of potassium in orthoclase solutions and in potassium-chlorid solutions*

Culture No.	Culture solution.	K_2O in solution.	Dry weight of plants.	K_2O in 100 plants.	K_2O increase over control.
		P. p. m.	*Gm.*	*Gm.*	*Per cent.*
1a	Control (distilled water)	0	1.51	0.0295	0
2a	Control with $CaSo_4$	0	1.53	.0281	− 4
3a	Orthoclase (solid phase present)	2 to 9	1.54	.0284	− 3
4a	Orthoclase with $CaSo_4$ (solid phases present)	2 to 9	1.58	.0272	− 13
1b	Control	0	1.36	.0365	0
2b	Control with $CaSo_4$	0	1.34	.0368	+ 1
3b	Orthoclase (solid phase present)	2 to 9	1.43	.0366	0
4b	Orthoclase with $CaSo_4$ (solid phases present)	2 to 9	1.45	.0372	+ 2
5b	K Cl	4	1.50	.0783	+114
6b	K Cl with $CaSo_4$	4	1.40	.0860	+136
1c	Control	0	3.68	.0310	0
2c	Orthoclase (solid phase present)	2 to 9	3.96	.0302	− 2
3c	Orthoclase with $CaSo_4$ (solid phases present)	2 to 9	3.68	.0345	+ 11
4c	Orthoclase with $CaCO_3$ (solid phases present)	2 to 9	3.92	.0341	+ 10
5c	Orthoclase with 50 p. p. m. NO_3 and 50 p. p. m. P_2O_5	2 to 9	3.64	.0341	+ 10
1d	Control	0	4.08	.0368	0
2d	Control with $CaSo_4$	0	4.48	.0395	+ 7
3d	Orthoclase (solid phase present), changed daily	2 to 9	4.30	.0457	+ 33
4d	Orthoclase and $CaSo_4$ (solid phases present), changed daily	2 to 9	4.50	.0411	+ 11
5d	Orthoclase (solid phase) and 4 p. p. m. K_2O as KCl, changed daily	6 to 13	4.50	.0978	+166
6d	KCl	4	4.45	.0947	+157
7d	KCl with $CaSo_4$	4	4.85	.1010	+175
8d	Orthoclase and KCl, changed once	6 to 13	4.20	.0683	+ 86
9d	Orthoclase, not changed	2 to 9	4.10	.0388	+ 5

AVAILABILITY OF POTASSIUM IN ORTHOCLASE SOLUTIONS NOT INCREASED BY LIME OR GYPSUM

The application of lime and gypsum to orthoclase-bearing soils has been considered by some workers as a means of increasing the availability of the potassium in such soils. The authors[1] found in an earlier investigation that the addition of lime or gypsum to orthoclase solutions containing the solid phase did not increase the concentration of the potassium in the solution. The data presented in Table I show that these substances also had no effect on the availability of the potassium in the orthoclase solution.

[1] BRIGGS, Lyman J., and BREAZEALE, J. F. AVAILABILITY OF POTASH IN CERTAIN ORTHOCLASE-BEARING SOILS AS AFFECTED BY LIME OR GYPSUM. *In* Jour. Agr. Research, v. 8, no. 1, p. 21–28. 1917.

AVAILABILITY OF THE POTASSIUM IN ORTHOCLASE SOLUTIONS NOT INCREASED BY BOILING THE SOLUTION

The effect of boiling an orthoclase solution on the subsequent availability of the potassium is shown in Table II. In this experiment the potassium content of the plants grown in the culture solution was compared with that of the original seed. The analyses show that within the errors of experiment the availability of the potassium was not modified by boiling the orthoclase solutions.

TABLE II.—*Effect of boiling orthoclase solutions on the availability of the soluble potassium*

Culture No.	Material analyzed.	K_2O in solution.	Dry weight of plants.	K_2O in 100 plants.	K_2O increase over control.
		P. p. m.	*Gm.*	*Gm.*	*Per cent.*
1	Original seed	.....	.....	0.0368	0
2	Seedlings grown in orthoclase solution (solid phase present).	2 to 9	4.00	.0386	+5
3	Seedlings grown in orthoclase solution (solid phase present), boiled	2 to 9	4.28	.0330	−8

AVAILABILITY OF POTASSIUM IN ORTHOCLASE SOLUTION NOT INCREASED BY PRESENCE OF CARBON DIOXID

Carbon dioxid is universally present in the soil solution. It is consequently desirable to determine whether the availability of the potassium in orthoclase may be measurably increased by the addition of carbon dioxid to the solution. A culture solution of orthoclase with the solid phase present was accordingly prepared, and a portion of this solution was saturated with carbon dioxid. Plants grown in the two solutions showed no difference in their potash content (Table III). It consequently appears that a weak acid, such as carbonic acid, in concentrations equivalent to those found in soil solutions, does not increase the availability of the potassium in orthoclase.

TABLE III.—*Effect of carbon dioxid on availability of potassium in orthoclase*

Culture No.	Culture solution.	K_2O in solution.	Dry weight of plants.	K_2O in 100 plants.	K_2O increase over control.
		P. p. m.	*Gm.*	*Gm.*	*Per cent.*
1	Orthoclase (solid phase present).	2 to 9	1.92	0.0284	0
2	Orthoclase (solid phase present) saturated with CO_2	2 to 9	1.72	.0284	0

SOLUBLE POTASSIUM IN ORTHOCLASE SOLUTIONS IS MADE AVAILABLE BY OXIDATION WITH ACIDS

To determine whether the soluble potassium in orthoclase could be available by oxidation with acids, the following experiment was carried out.

Finely ground orthoclase was added to about 100 liters of water, and this mixture was shaken at intervals until equilibrium was established and the maximum solubility of the potassium in the feldspar had been obtained.

One-half of this solution was filtered through a padded folded paper filter, and the clear solution, together with a few cubic centimeters of a mixture of hydrochloric and nitric acids, was then evaporated to dryness in Jena beakers. The excess of acids was driven off, and the solution was brought back to volume with purified distilled water. A little calcium carbonate ($CaCO_3$) was then added to insure alkalinity. Wheat seedlings were grown in such cultures for 14 days, the solutions being changed daily. The results are given in Table IV, series a.

TABLE IV.—*Effect of oxidation of soluble potassium in orthoclase on its availability*

Culture No.	Culture solution.	K_2O in solution	Dry weight of plants.	K_2O in 100 plants	K_2O increase over control
		P. p. m.	*Gm.*	*Gm.*	*Per cent.*
1a	Control	0	2.42	0.0326	0
2a	Orthoclase (solid phase present)	2 to 9	2.52	.0349	−7
3a	Orthoclase solution filtered and evaporated with acids	4	2.88	.0722	+121
4a	KCl	5	2.48	.0620	+90
1b	Control	0	3.30	.0203	0
2b	Orthoclase (solid phase present)	2 to 9	2.66	.0180	+11
3b	Orthoclase solution filtered and evaporated with acids	4	3.30	.0357	+76
4b	KCl	5	3.36	.0815	.....

The wheat seedlings grown in orthoclase solutions in which the potassium compounds had been oxidized showed a total potash content at the end of the experiment about twice that of the plants grown in distilled water. On the other hand, the plants grown in the untreated orthoclase solution showed as before no gain in potash over the control.

A repetition of the experiment, Table IV, series 6, again showed a marked increase in the potash content of the plants grown in the solutions prepared from the oxidized solute. The orthoclase solution used in this series of experiments had stood in contact with the powdered mineral for about 2 months, being shaken at frequent intervals. The experiment extended over 19 days, the culture solutions being changed daily.

It is of interest to note that in the first series of experiments the potassium absorbed from the oxidized solute was equal to that absorbed from a potassium-chlorid solution containing 5 parts per million of potassium oxid. In the second series, the plants grown in the potassium-chlorid solution showed relatively a marked increase in their potassium content.

INCREASED AVAILABILITY OF POTASH IN OXIDIZED ORTHOCLASE SOLUTIONS NOT DUE TO ACTION OF ACIDS ON SUSPENDED COLLOIDS

The orthoclase solutions used in the preceding experiments contained some suspended colloidal material. It is therefore possible that the observed increase in the availbility of the potassium may have resulted from the direct action of the acids on the suspended colloids. To determine this point, a saturated solution of orthoclase was prepared and filtered through a Pasteur-Chamberland tube. A part of this filtrate was then treated with acids and evaporated to dryness, as described above, and subsequently diluted to its original volume and used as a culture solution. A portion of the original orthoclase solution which had not received the acid treatment was used as a control. The results of two experiments, made at different times, are given in Table V.

TABLE V.—*Effect of freeing culture solutions from colloids*

Culture No.	Culture solution.	K_2O in 100 plants.	K_2O increase over control.
		Gm.	*Per cent.*
1a....	Orthoclase solution, untreated with acids.....	0.0272	0
2a....	Orthoclase solution, treated with acids.....	.0597	+120
1b....	Orthoclase solution, untreated with acids.....	.0302	0
2b....	Orthoclase solution, treated with acids.....	.0551	+83

The analyses of the plants show as before a marked gain in the potassium content of the plants grown in the acid-treated solutions. The colloids can not in this case be considered the source of the potash made available by the acid treatment, since the colloidal material was removed from the solution before the acids were added. We are consequently led to conclude that the orthoclase solutions contain potassium in true solution (as distinguished from colloidal suspension) and that the potassium is chemically combined in such a manner that it is not available to plants.

DISCUSSION

The failure of wheat seedlings to absorb the potassium found by analysis in orthoclase solutions suggests that the potassium is combined with other elements in a slightly soluble molecular complex. This is supported by the fact that the potassium may be made available by treatment with strong acids, which would result from the breaking

down of the complex. We may also assume that the solute complex is not dissociated, at least in such a way as to liberate potassium ions. For we can say with some assurance that free potassium ions would be absorbed by the wheat seedlings. We have evidence of this in the selective absorption exercised by wheat seedlings on potassium-chlorid solutions in which the potassium (either as $\overset{+}{K}$ or $\overset{+}{K}\overset{-}{OH}$) is selectively absorbed to such an extent that the culture solution becomes distinctly acid.

The effect of the oxidation of the solute complex in orthoclase solutions by hydrochloric and nitric acids is to reduce the potassium in the complex to potassium chlorid or potassium nitrate (KNO_3), in which form it dissociates and is readily absorbed.

The evidence presented in the case of orthoclase leads to the general statement that the concentration of a specific plant food element in the soil solution does not necessarily provide any measure of its availability. The question of availability must be referred to the plant itself, except perhaps in those cases in which the element in question is known to be ionized.

The results of our experiments have an immediate bearing on various investigations now in progress looking toward the utilization of orthoclase as a source of potash. It should be borne in mind that the application of finely ground orthoclase, without other treatment, probably does not contribute immediately to the available potash content of the soil.

CONCLUSIONS

From the experimental data presented the following conclusions are drawn, subject to the limitations imposed by the experimental error:

(1) The soluble potassium in aqueous solutions derived from finely ground orthoclase is not absorbed by wheat seedlings to a measurable degree.

(2) The availability of the potassium is not increased by the addition of lime, gypsum, or carbon dioxid to the solutions or by boiling the solutions.

(3) The soluble potassium in orthoclase solutions is made available by oxidizing the solute with hydrochloric and nitric acids.

(4) The increase in the availability following oxidation is not due to the action of the acids on suspended colloids, but is to be ascribed to the breaking down of the complex solute molecule.

(5) The concentration of a specific plant food element in the soil solution does not necessarily provide any measure of its availability. The question of availability must be referred to the plant itself.

COMPOSITION OF TUBERS, SKINS, AND SPROUTS OF THREE VARIETIES OF POTATOES

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PREVIOUS INVESTIGATIONS

The composition of the potato undoubtedly varies with the soil and with the fertilizer used, as well as with other environmental and climatic conditions. Since the sprouts depend for their growth on the tubers, the composition of the tubers may influence that of the sprouts to no small extent.

The composition of tubers from different varieties of potato plants has not been investigated, nor has any extended study been made of the composition and growth changes of sprouts from the same or different varieties of tubers. Buckner (*3*),[1] who has reported analyses of sprouts skins, and tubers from one variety of potatoes for ash, phosphoric acid, magnesium oxid, calcium oxid, and silica dioxid found a relatively high percentage of ash in the sprouts.

The cause and regulation of rest periods in plants have been studied for years, several investigations having been devoted to the effect of various chemicals on tubers, with a view to shortening the rest period. Experiments at the Arizona Agricultural Experiment Station (*5*) have shown that ethyl bromid, carbon tetrachlorid, ammonia, gasoline, ethyl chlorid, and bromin are effective in bringing dormant tubers into activity—that is, in stimulating the buds. Seed tubers treated with manganese chlorid and ethyl ether showed no differences in the growth of foliage but exhibited a pronounced increase of tuber formation. Müller (*6*) claims to have shortened the rest period of tubers by storing them for one month at 0° C. Appleman (*1*) has found an increase of both total and reducing sugar in tubers stored at 0° C. According to this investigator, the carbohydrate transformation during the rest period depends entirely on the changing temperature. He has separated also the nitrogenous and the phosphorus compounds of tubers stored for various periods.

Schulze and Barbieri (*9*), in 1878, showed that potato sprouts contained nonprotein nitrogen in addition to protein nitrogen and found asparagin and solanin. It was shown that the potato contained 0.38 per cent nitrogen, practically one-half, or 0.18 per cent of which was in

[1] Reference is made by number (italic) to "Literature cited," p. 634–635.

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the form of protein nitrogen. Eighty-one per cent of the nitrogen in the tubers proved to be soluble—that is, appeared in the pressed juice of the potato. The sprouts contained 1.5 per cent of nitrogen on a dry basis. In 1880 these same investigators (*10*) isolated leucin and tyrosin from an alcoholic extract of potato sprouts. Osborne and Campbell (7) obtained a globulin called "tuberin," the properties of which they describe, and a small amount of another protein from potato. Sjollema and Rinkes (*11*) have studied the hydrolysis of potato protein. They precipitated the protein with a saturated sodium-chlorid solution, dissolved it in 10 per cent sodium chlorid, dialyzed it to remove the salt, and finally reprecipitated it with alcohol. The nitrogen content of the protein obtained was 14.9 per cent. Their investigation was divided as follows: (1) Estimation of the various diamino acids (Van Slyke method); (2) hydrolysis of protein by hydrofluoric acid; (3) estimation of different diamino acids (Kossel and Patten method); (4) estimation of momoamino nitrogen by Fisher's esterfication method; and (5) estimation of tyrosin. The result of their study of the hydrolysis of potato protein showed that 100 gm. contained nitrogenous substances distributed as follows:

	Gm.		Gm.
Ammonia	1.8	Alanin	4.9
Histidin	2.3	Leucin	12.2
Arginin	4.2	Valin	1.1
Lysin	3.3	Valin and alanin	8.2
Cystin	4.4	Valin and leucin	1.9
Glutaminic acid	4.6	Phenylalanin	3.9
Prolin	3.0	Tyrosin	4.3

Ramsay and Robertson (*8*) have reported data on the rate of assimilation of food from the soil by the potato plant and the relative proportion of each of the principal elements contained in the plants.

The fact that Bordeaux-sprayed potato plants in certain localities give larger yields of tubers than unsprayed plants has been established by a series of experiments extending over many seasons at the Vermont, Maine, and New York Agricultural Experiment Stations. Stewart, Eustace, and Sirrine (*12*), of the New York Experiment Station, reported several years ago that one lot of Bordeaux-sprayed tubers was higher in solids and starch than a corresponding lot of unsprayed tubers. Charles D. Woods, of the Maine Experiment Station, has reported similar findings (*13*). The writer has analyzed several samples of Bordeaux-sprayed and unsprayed tubers grown in Maine during the past three seasons, generally finding a higher content of solids and nitrogen in the sprayed than in the unsprayed tubers.

OBJECT OF PRESENT INVESTIGATION

It was thought that some variation in the composition of sprouts of the same or different varieties of tubers might be found. It was also believed that the copper sprays used to control *Phytophthora infestans*, or late blight of the potato, might influence the time of sprouting—that is, increase or decrease the rest period compared with that of the unsprayed tubers—or that these sprays might modify the composition of the sprouts of the same varieties of tubers, just as copper sprays apparently influence the composition of the tubers. An investigation, therefore, was undertaken to determine, if possible, whether any of the changes just mentioned took place and to secure data on the chemical composition of sprouts, skins, and tubers.

EXPERIMENTAL WORK

DESCRIPTION OF SAMPLES

In the course of some tests on the influence of copper sprays on the control of *Phytophthora infestans*, or late blight of the potato plant, and on the yield of the tubers, several samples of Maine and Connecticut tubers dug in September, 1918, were stored in the laboratory at Washington, D. C., from October, 1918, until they were analyzed in the spring of 1919. Samples of Rural New Yorker (No. 12), Green Mountain (No. 15), and Irish Cobbler tubers (No. 9) from Maine, from selected hills where the vines were vigorous and healthy, as well as Green Mountain tubers (No. 3 and 6) from Connecticut, taken from portions of the plots which stayed green the longest were used for these tests. All the tubers were held in a dark closet at laboratory temperature (average 70° F.) from October, 1918, until February, April, or June, 1919. This relatively high temperature may have affected the composition of both tubers and sprouts. Several sprouts developed on each tuber, those on the Rural New Yorker appearing later than those on the Green Mountain and Irish Cobbler tubers. The sprouts of the Rural New Yorker tubers were short and thick, while those of the Green Mountain and Irish Cobbler tubers were comparatively long and branching.

METHODS OF ANALYSIS

At the time of analysis the sprouts were removed from the tubers and sifted to free them from adhering dirt. The tubers were washed and dried and then pared as thin as possible, a difficult matter because of their soft condition. The weights of the moist skins, tubers, and sprouts were taken separately. The tubers and the skins were then ground separately in a meat grinder, and each sample was well mixed and placed in a Mason jar with rubber and top. The sprouts were placed in a stoppered bottle. The analyses were begun as soon as possible. Solids, ash, phosphorus, and nitrogen determination were made on the moist samples,

the methods of the Association of Official Agricultural Chemists (2) being used.

Water extracts of the sprouts, skins, and tubers were prepared by macerating 50 gm. of the moist samples with a pestle in a mortar, then rinsing the material into a graduated flask with water, adding 10 cc. of toluene and making up to 500 cc. with water. The flasks were shaken each minute for the first 5 minutes and then every 15 minutes for the first hour, after which they stood overnight at room temperature. The next morning the liquid was removed with a pipette and filtered through glass wool and then through filter paper. The following determinations were made on the water extracts: (1) Soluble nitrogen, employing 25 cc.; (2) soluble phosphoric acid, employing 50 cc.; (3) ammonia nitrogen, employing 5 cc., by the aeration method of Folin (14) and nesslerizing the volatile nitrogen; (4) separation of nitrogenous compounds, employing 100 cc.

In making a separation of the nitrogenous compounds, 100 cc. of the solution were acidified and heated to boiling. The coagulable protein was removed first, then the remaining protein, by precipitation with dilute lead acetate solution. The lead was removed from the filtrate with hydrogen sulphid, the lead sulphid being filtered off and washed with a dilute solution of hydrochloric acid through which hydrogen sulphid had been passed. The solution containing the amino acids, amids, etc., was then made to volume, and the total nitrogen was determined in an aliquot. The largest portion of the filtrate was precipitated with phosphotungstic acid according to the Hausman method, and the nitrogen in the filtrate (monoamino and amid nitrogen) was determined. The nitrogen of the diamino acids and other bases was obtained by difference.

Copper was determined in certain of the samples by the colorimetric method, using potassium ferrocyanid and standard solutions of copper sulphate. This method, which has been shown to yield identical results with the electrolytic method, has the advantage of giving accurate results when minute amounts of copper are present and of being applicable when the electrolytic method is not.

RESULTS OF ANALYSIS

RELATIVE WEIGHTS OF SPROUTS AND TUBERS

The samples of sprouts, skins, and tubers numbered 1 to 3 and 4 to 6 (Table I) were of the Green Mountain variety. On February 1, 1919, the sprouts on these two sets of tubers constituted 4.6 per cent of the total moist weight of sprouts, skins, and tubers. Samples 1, 2, and 3 were from vines sprayed with 5–5–50 Bordeaux spray, while samples 4, 5, and 6 were from unsprayed vines. Samples 7, 8, and 9 (sprouts, skins, and tubers) were from Irish Cobbler plants. At the time of analysis,

April, 1919, the sprouts constituted 13.33 per cent of the total moist weight of sprouts, skins, and tubers. These plants had been sprayed with 5–5–50 Bordeaux.

The Rural New Yorker tubers (samples 10, 11, and 12) and the Green Mountain tubers (samples 13, 14, and 15) were grown at Foxcroft, Me., and had been sprayed with 5–5–50 Bordeaux spray. At the time of analysis, April, 1919, the sprouts of the Rural New Yorker tubers, a late variety, constituted 3.5 per cent and the Green Mountain sprouts 7.2 per cent of the total moist weight. These two varieties of tubers, dug from the same field late in September, 1918, were stored in the laboratory under identical conditions.

Samples of Green Mountain potatoes from Connecticut (samples 18 and 19), as well as the Irish Cobbler tubers grown in Maine (sample 21), were held at laboratory temperature until June, 1919, when the sprouts and tubers were analyzed separately. While the sprouts of the Irish Cobblers were large and fresh, those of the two samples of Green Mountain potatoes were partially dried and withered. No analyses of the skins were made for these three samples analyzed in June because of the difficulty of paring the soft tubers.

The variations in the percentage composition of sprouts obtained from tubers stored under identical conditions can be explained only on the basis of the presence in varying amounts of growth-promoting substances in the different varieties of tubers.

COMPOSITION OF SPROUTS AND TUBERS

The analytical data in Table I include the distribution of nitrogen in terms of total nitrogen. The total weight and percentage distribution of the ash, phosphoric acid, and nitrogen compounds present in the sprouts and tubers are given in Table II. Table III shows the ash, phosphoric acid, and nitrogen results on a water-free basis.

TABLE I.—*Analyses of tubers, skins, and sprouts on moist basis*

Source of potatoes and date of analysis.	Sample No.	Description of material.	Solids.	Ash.	P_2O_5.	Water-soluble P_2O_5.	Water-soluble P_2O_5 as total P_2O_5.	Total nitrogen.	Water-soluble nitrogen.	Coagulable nitrogen.	Nitrogen precipitated by lead acetate.	Non-protein nitrogen.	Amid and mono-amino nitrogen.	Diamino and other bases nitrogen.	Free NH_2 nitrogen.	Distribution of nitrogen in total nitrogen.				
																Water-soluble.	Protein.	Amid and mono-amino.	Diamino and other bases.	Free NH_3.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Connecticut, February, 1919.	1	Sprouts, Green Mountain, sprayed.	19.14	1.62	0.43	0.33	76.74	0.76	0.44	0.033	0.041	0.365	0.291	0.074	0.0041	57.89	51.97	38.29	9.74	0.54
	2	Skins, Green Mountain, sprayed.	26.38	1.45	.24	.14	58.33	.50	.36	.080	.012	.267	.203	.064	.0062	72.00	46.52	40.60	12.80	1.24
	3	Tubers, Green Mountain, sprayed.	24.29	1.15	.24	.14	58.33	.45	.42	.076	.020	.322	.239	.083	.0042	93.33	28.45	53.11	18.44	.93
	4	Sprouts, Green Mountain, unsprayed.	18.62	1.54	.43	.33	76.74	.74	.39	.033	.039	.329	.284	.045	.0041	52.70	55.54	38.38	6.08	.55
	5	Skins, Green Mountain, unsprayed.	23.13	1.44	.23	.14	60.87	.41	.31	.070	.014	.228	.200	.028	.0094	75.61	44.39	48.78	6.83	2.29
	6	Tubers, Green Mountain, unsprayed.	21.75	1.12	.21	.13	61.90	.40	.37	.063	.016	.291	.227	.064	.0036	92.50	27.25	56.75	16.00	.90
Maine, April, 1919.	7	Sprouts, Irish Cobbler, sprayed.	19.24	1.42	.33	.20	60.61	.73	.44	.059	.031	.358	.286	.072	.....	60.27	50.96	39.18	9.86	.....
	8	Skins, Irish Cobbler, sprayed...	33.54	1.52	.25	.14	56.00	.67	.44	.103	013	.335	.176	.159	.0037	65.67	50.00	26.27	23.73	.55
	9	Tubers, Irish Cobbler, sprayed..	32.14	1.17	.24	.14	58.33	.63	.49	.076	.010	.388	.280	.108	.0028	77.78	38.41	44.44	17.14	.44
	10	Sprouts, Rural New Yorker, sprayed.	21.55	1.41	.37	.22	59.46	.75	.43	.038	.035	.359	.312	.047	.0040	57.33	52.13	41.60	6.27	.53
	11	Skins, Rural New Yorker, sprayed.	33.77	1.74	.27	.15	55.56	.43	.24	.056	.008	.187	.118	.069	.0032	55.81	56.51	27.44	16.05	.75
	12	Tubers, Rural New Yorker, sprayed.	34.01	1.29	.25	.12	48.00	.39	.30	.056	.008	.238	.164	.074	.0017	76.92	38.98	42.05	18.97	.44
	13	Sprouts, Green Mountain, sprayed.	18.57	1.38	.40	.25	62.50	.72	.44	.031	.034	.350	.302	.048	.0045	61.11	51.39	41.94	6.67	.63
	14	Skins, Green Mountain, sprayed.	31.97	1.52	.24	.14	58.33	.42	.25	.066	.011	.180	.134	.046	.0040	59.52	57.15	31.90	10.95	.95
	15	Tubers, Green Mountain, sprayed.	32.23	1.25	.23	.12	52.17	.35	.29	.068	.006	.208	.135	.073	.0025	82.86	40.57	38.57	20.86	.71
	16	Sprouts,[1] Green Mountain, sprayed.	32.93	3.28	.53	.36	67.92	1.27	.72	.029	.....	.63	.53	.10	.013	56.69	50.40	41.73	7.87	1.00

Maine and Connecticut, June, 1919...	17	Sprouts,[1] Green Mountain, unsprayed.	27.35	2.83	.56	.31	55.36	1.17	.65	.033	.....	.58	.49	09	.005	55.56	50.43	41.89	7.69	.43
	18	Tubers,[1] Green Mountain, sprayed.	30.15	1.55	.25	.10	40.00	.70	.57	.042	.....	.50	.46	.04	.005	81.43	28.57	65.71	5.71	.14
	19	Tubers.[1] Green Mountain, unsprayed.	30.66	1.54	.23	.13	56.52	.65	.52	.042	.....	.46	.39	.07	.001	80.00	29.23	60.00	10.77	.15
	20	Sprouts,[2] Irish Cobbler, sprayed	28.32	2.41	.52	.25	48.08	1.10	.66	.049	.....	.54	.49	.05	.003	60.00	50.90	44.55	4.55	.30
	21	Tubers,[2] Irish Cobbler, sprayed	35.88	2.02	.28	.13	46.43	.74	.51	.049	.....	.41	.32	.09	.022	68.92	44.60	43.24	12.16	2.97

[1] Sprouts and tubers partly dried, analyzed in June; tubers from same lot as sample No. 3 and 6 analyzed in February.
[2] Sprouts fresh and tubers dried, analyzed in June; tubers from same lot as sample No. 9 analyzed in April.

TABLE II.—*Weights, percentages, and chemical constituents of tubers, skins, and sprouts*

Sample No.	Description of material.	Moist weight.	Percentage weight of sprouts in total tubers.	Dry weight.	Ash.	Ash in solids.	P_2O_5.	P_2O_5 in solids.	P_2O_5 in ash.	Nitrogen.	Total nitrogen.	Total ash.	Total P_2O_5.	Copper, dry, basis.
		Gm.		*Gm.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Per cent.*	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*P. p. m.*
1	Sprouts, Green Mountain, sprayed	73	4.63	13.97	1.18	8.45	0.25	1.80	21.19	0.55	7.20	5.80	7.99	.....
4	Sprouts, Green Mountain, unsprayed	65.5	4.60	12.20	1.01	8.28	.21	1.72	20.80	.48	7.99	5.64	8.08	.....
7	Sprouts, Irish Cobbler, sprayed	231.5	13.33	44.54	3.29	7.39	.76	1.71	23.10	1.69	14.81	14.32	17.16	40
10	Sprouts, Rural New Yorker, sprayed	76	3.50	16.38	1.07	6.54	.28	1.71	26.17	.57	6.32	3.44	4.95	.....
13	Sprouts, Green Mountain, sprayed	101	7.19	18.76	1.39	7.41	.40	2.13	28.78	.73	13.04	7.36	11.63	41
2	Skins, Green Mountain, sprayed	632	.....	166.70	9.16	5.50	1.23	.74	13.43	3.13	.....	.....	.....	14
5	Skins, Green Mountain, unsprayed	531	.....	122.82	7.64	6.22	.98	.80	12.83	2.20	.....	.....	.....	21
8	Skins, Irish Cobbler, sprayed	592	.....	198.55	9.00	4.53	1.48	.70	16.44	3.97	.....	.....	.....	.....
11	Skins, Rural New Yorker, sprayed	660	.....	222.88	11.48	5.15	1.78	.80	15.51	2.84	.....	.....	.....	.....
14	Skins, Green Mountain, sprayed	443	.....	141.62	6.73	4.75	1.06	.75	15.75	1.86	.....	.....	.....	.....
3	Tubers, Green Mountain, sprayed	871	.....	211.56	10.02	4.74	1.65	.80	16.47	3.96	51.83	49.12	52.71	30
6	Tubers, Green Mountain, unsprayed	828	.....	180.09	9.27	5.15	1.41	.80	15.21	3.33	55.41	51.73	54.23	18
9	Tubers, Irish Cobbler, sprayed	913	.....	293.43	10.68	3.64	2.19	.75	20.51	5.75	50.39	46.39	49.44	.....
12	Tubers, Rural New Yorker, sprayed	1,439	.....	489.26	18.56	3.79	3.60	.74	19.39	5.61	62.20	59.66	63.60	.....
15	Tubers, Green Mountain, sprayed	861	.....	277.50	10.76	3.88	1.98	.71	18.40	3.01	53.75	56.99	57.56	.....
16	Sprouts, Green Mountain, sprayed	66.5	5.22	21.90	2.18	9.96	.35	1.60	16.06	.84	.....	.....	.....	.....
17	Sprouts, Green Mountain, unsprayed	56	5.64	15.32	1.58	10.31	.31	2.02	19.62	.66	.....	.....	.....	.....
20	Sprouts, Irish Cobbler, sprayed	80.5	17.06	22.80	1.94	8.50	.42	1..84	21.65	.89	.....	.....	.....	.....

TABLE III.—*Percentage of ash, phosphoric acid, and nitrogen in potato sprouts and tubers on water-free basis*

Source of potatoes and date of analysis.	Sample No.	Description of material.	Ash.	P_2O_5.	Nitrogen.	Condition of samples.
			Per cent.	*Per cent.*	*Per cent.*	
Connecticut, February, 1919	1	Sprouts, Green Mountain, sprayed	8.46	2.25	3.97	Sprouts fresh.
	4	Sprouts, Green Mountain, unsprayed	8.27	2.31	3.97	Do.
Maine, April, 1919	7	Sprouts, Irish Cobbler, sprayed	7.38	1.72	3.79	Do.
	10	Sprouts, Rural New Yorker, sprayed	6.54	1.72	3.48	Do.
	13	Sprouts, Green Mountain, sprayed	7.43	2.15	3.88	Do.
Connecticut, June, 1919	16	....do	9.96	1.61	3.86	Sprouts withered.
	17	Sprouts, Green Mountain, unsprayed	10.35	2.05	4.28	Do.
Maine, June, 1919	20	Sprouts, Irish Cobbler, sprayed	8.51	1.84	3.88	Sprouts fresh.
Connecticut, February, 1919	3	Tubers, Green Mountain, sprayed	4.73	.98	1.85	Tubers soft.
	6	Tubers, Green Mountain, unsprayed	5.15	.97	1.84	Do.
Maine, April, 1919	9	Tubers, Irish cobbler, sprayed	3.64	.75	1.96	Do.
	12	Tubers, Rural New Yorker, sprayed	3.79	.74	1.15	Do.
	15	Tubers, Green Mountain, sprayed	3.88	.71	1.09	Do.
Connecticut, June, 1919	18	....do	5.14	.83	2.32	Tubers withered and very soft.
	19	Tubers, Green Mountain, unsprayed	5.02	.75	2.12	Do.
Maine	21	Tubers, Irish Cobbler, unsprayed	5.63	.78	2.06	Do.

SOLIDS.—The solids of the young sprouts of the Green Mountain and Irish Cobbler varieties, samples 1, 4, 7, and 13, were exceedingly uniform, notwithstanding variations in the water content of the tubers. The moisture content of the sprouts seemed to be maintained at the expense of the tubers. The Rural New Yorker sprouts, sample 10, contained more solids than the other young sprouts. The older, partly dried sprouts, samples 16, 17, and 20, were highest in solids. The moisture content of the different varieties of tubers decreased with the period of standing in the laboratory.

ASH.—The important feature of the ash analyses was the high percentage of ash in the sprouts as compared with that in the tubers, made more evident on calculating the results to a moisture-free basis. The skins showed a higher percentage of ash than the tubers. The sprouts showed a selective action and withdrew the ash from the tubers in a greater proportion than it originally existed in them, so that the percentage of ash in the solids was nearly twice as high for the sprouts as for the tubers. A higher percentage of ash was found in the old than in the young sprouts and tubers.

PHOSPHORIC ACID (P_2O_5).—The phosphoric acid content of the sprouts was greater than that of the skins or tubers. In the solids of the sprouts it averaged 1.81 per cent and was less than 1 per cent for the skins and tubers. In the ash of the sprouts it varied from 20 to 30 per cent, while it was less than 20 per cent in the ash of the tubers and skins. From 60 to 76 per cent of the total phosphoric acid content of the young sprouts was water-soluble as compared with but 50 to 60 per cent of the phosphoric acid content of the skins and tubers. Somewhat less phosphoric acid was water-soluble in the older sprouts and tubers than in the younger samples.

NITROGEN.—The nitrogen content of the sprouts was apparently uniformly maintained. In the five samples of young sprouts examined (No. 1, 4, 7, 10, and 13) approximately 0.75 per cent of nitrogen was found. The older sprouts contained from 1.10 to 1.27 per cent nitrogen. The different varieties of sprouts showed a uniform percentage of the total nitrogen, both as protein nitrogen and as amid and monoamino nitrogen. The amid and monoamino nitrogen formed about 40 per cent and the diamino and other basic nitrogen formed less than 10 per cent of the total nitrogen of the sprouts. A higher percentage of amid and monoamino nitrogen was found in the older Green Mountain sprouts (samples 16 and 17) than in the younger Green Mountain sprouts (samples 1 and 4). The sprouts contained a lower percentage of total nitrogen in the form of coagulable protein but a higher percentage as total protein than did the tubers. The younger sprouts also contained a lower percentage of the total nitrogen as amid and monoamino nitrogen and of diamino and other base nitrogen than did the tubers. Based on the

percentage of total nitrogen, the younger tubers showed a greater content of water-soluble nitrogen than the older tubers. The samples of tubers analyzed in June contained a larger amount of total nitrogen than those analyzed earlier because of the added loss in water and the reduction in sugar and starch of the tubers caused by respiration.

AMMONIA.—The amount of free ammonia in the young sprouts was constant. More ammonia was found in the skins than in the tubers or sprouts. The older tubers apparently contained less ammonia than the younger ones.

COPPER.—All the samples tested showed copper, the sprouts containing somewhat more than the tubers or skin.

FACTORS WHICH MAY INFLUENCE THE COMPOSITION OF POTATO SPROUTS

Numerous factors may influence the composition of potato sprouts. Excluding the various physiological and other diseases, a few of these factors may be mentioned briefly.

VARIETY.—The analyses indicate that the composition of sprouts of the same age from the three different varieties of tubers examined was uniform. This was true in spite of the fact that the sprouts formed varying percentages of the total moist weight of tubers, skin, and sprouts and contained varying percentages of the total nitrogen, phosphoric acid, and ash.

BORDEAUX SPRAYING.—The results for solids and ash on the Green Mountain sprouts, skins, and tubers from sprayed vines (samples 1, 2, and 3) were slightly higher than those on sprouts, skins, and tubers from corresponding unsprayed vines (samples 4, 5, and 6). The distribution of the nitrogenous substances showed the same general trend in the two samples. The tubers from both the sprayed and unsprayed plants formed sprouts with equal rapidity, judging by the percentage weights of sprouts and tubers. The sprouts constituted 4.63 and 4.59 per cent of the total weight of sprouts, skins, and tubers of the two samples at the time of analysis. The percentages of nitrogen, phosphoric acid, and ash removed by the sprouts in the two cases were remarkably uniform. While it is impossible to draw a definite conclusion from the analyses of two samples only, the indication from these and other samples is that the percentage of solids and nitrogen is higher in the tubers from sprayed than in those from unsprayed potato vines.

SOIL, CLIMATE, AND FERTILIZER.—The potato is no exception to the well-known fact that soil, climate, fertilizer, and other factors often influence the composition of the crop. Calculated to a water-free basis (Table III), the Connecticut tubers and sprouts gave higher results for ash, phosphoric acid, and nitrogen than the other samples, suggesting an influence of soil and climate on the composition of the potato.

AGE AND GROWTH.—The age of the sprout apparently influences its composition. A higher percentage of solids and ash was found in the

older than in the younger sprouts. Many changes in the percentage of water-soluble to total phosphorus and in the distribution of the nitrogenous substances follow the growth of the sprouts. The principal period of growth of the sprouts under the conditions of this test occurred during the period up to March, or from 60 to 150 days after the tubers had been dug. From 150 days until the end of June, or 270 days after digging, the increase in weight of the sprouts was less. The sprouts of the Irish Cobbler tubers analyzed in June (sample 20) constituted 17 per cent, while those of the Green Mountain tubers (samples 16 and 17) constituted 5.5 per cent of the total weight of tubers and sprouts. The Cobbler is an early potato and the Green Mountain a late one. Both varieties had reached their limit of sprouting in June under the conditions of these tests. Apparently the growth-promoting principle is much more active or is present in larger amounts in the Irish Cobbler than in the Green Mountain and Rural New Yorkers.

DISTRIBUTION OF NITROGEN, PHOSPHORIC ACID, AND ASH IN SPROUTS, SKINS, AND TUBERS

The percentage distribution of nitrogen, phosphoric acid, and ash in sprouts, skins, and tubers depends upon the relative weights of sprouts and tubers. Although the sprouts of the Rural New Yorker tubers constituted 3.5 per cent of the total moist weight of tubers, skins, and sprouts, they contained 6.32 per cent of the total nitrogen. The sprouts of the Irish Cobbler on the same date constituted 13.33 per cent of the total moist weight and contained 14.81 per cent of the total nitrogen. Similar ratios hold for the distribution of phosphoric acid and ash. This indicates that the sprouts obtained the nitrogen, phosphoric acid, and ash in certain proportions from the tubers, the tubers simply acting as reservoirs for the sprouts. The action of the sprouts was selective, as might be expected in young, growing tissue. The solids of the sprouts contained 4 per cent of nitrogen, while the solids of the tubers and skins contained less than 2 per cent. In the Irish Cobbler the percentage of ash, phosphoric acid, and nitrogen remaining in the tubers after sprouting had ceased was less than 50 per cent of the total.

Buckner (*3*) found 17.77 per cent of the total phosphoric acid in the sprouts and 67.13 per cent in the exhausted tubers. Because he found that 50 per cent or more of the mineral matter was left in the tubers, he thought that a large amount of ash was necessary to bring about the katabolic changes involved in sprouting. He obtained the following results:

Material examined.	Ash in solids.	P_2O_5 in ash.	CaO in ash.	MgO in ash	K_2O in ash.	SiO_2 in ash.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
New sprouts	9.91	12.56	0.90	2.72	40.40	0.95
Skins	8.14	5.89	1.70	1.33	40.33	8.45
Tubers	4.37	12.4	.75	2.25	53.52	.60

Chlorin and other ash constituents in potato ash are not included in Buckner's analyses.

SUMMARY

Analytical data for sprouts, skins, and tubers of three varieties of Bordeaux-sprayed potatoes stored at labratory temperature (average 70° F.) showed little variation in composition for the different varieties, the age of the sprout apparently influencing the composition more than the variety. Data for Green Mountain sprouts, skins, and tubers from Bordeaux-sprayed and from unsprayed plants indicated that the spray did not change the rate of growth or the composition of the sprouts.

Biological changes are taking place in the formation and growth of the sprouts. The percentage distribution of the nitrogenous substances showed the sprouts to contain more protein and less diamino and other basic nitrogen than the skins and tubers. The sprouts showed a selective action in withdrawing from the tubers nitrogen, ash, phosphoric acid, and water in larger proportion than was originally present.

The sprouts remained fresh and continued to grow as long as any water was available in the tubers. The sprouts of the Irish Cobbler tubers constituted 17 per cent of the total weight of the sprouts and tubers at the time the tubers were exhausted, while the Green Mountain sprouts, under the same conditions, constituted 5.5 per cent of the total weight. An increased concentration or activity of the growth-promoting agent or agents in Irish Cobbler tubers is suggested.

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FURTHER STUDIES IN THE DETERIORATION OF SUGARS IN STORAGE[1]

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In a study of the deterioration of Cuban raw sugars stored under normal conditions during the summer of 1919 certain conclusions were indicated concerning the correlation between chemical and bacteriological analysis, with special reference to losses in sucrose content.[2] It was shown that the keeping quality of a sugar depends not only upon the moisture ratio but likewise upon the content of microorganisms and that any prediction concerning deterioration involves a concomitant consideration of these two factors.[3] In the present investigation of sugars stored in 1920 the technic and procedure were identical with those previously used, which have been described elsewhere;[4] the only difference was that in 1920 the position of the bags in any single pile was reversed after four weeks' incubation to obtain uniformity of environment, and the bags were placed on scantling 1 foot from the floor and were protected by a covering of a single layer of sacks.

It was especially designed to have under observation as large a variety of sugars as possible, and from the succeeding data it will be seen that all extremes in polarization, moisture, and number of microorganisms are to be found. This is not only true of the different marks chosen but more significantly of the bags of each mark. As a rule 3 bags which varied sufficiently to be considered representative of the mark were chosen, and in some instances, where the variations in a lot were unusual, 6 bags were taken. It may be mentioned parenthetically that it was planned to sample the bags monthly for six months, but because of the postponed arrival of sugar it was necessary to delay the initial sampling and thus curtail the number of analyses. In the succeeding tables the names of the marks have been abbreviated to symbols, since there has been no intention of subjecting any of the sugars to criticism. All the sugars came from Cuba with the exception of 2 marks, M and A, from Porto Rico. Seven of the 10 marks represent sugars transported by vessel, the remaining 3 (Am, O, and Phil) having come by railroad via

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Key West. The following numbers of bags of each mark were received: F, 2,303; Port, 4,694; Cun, 17,000; Agr, 13,500; Cab, 10,000; Am, 2,831; O, 1,406; Pil, 4,060; M, 11,300; Ag, 5,000—totaling 23,070,080 pounds of sugar. The bags under observation were chosen from among these.

In Table I are presented the chemical and bacteriological analyses of the sugars under normal storage. The moisture ratio or factor of safety has been calculated according to the formula $\text{M. R.} = \frac{\text{Moisture}}{100 - \text{polarization}}$, a detailed discussion of which may be found in previous publications.[1] The last column refers to the percentage of molds based on the total number of microorganisms per gram.

TABLE I.—*Chemical and bacteriological analyses of Cuban raw sugars in storage*[2]

TRANSPORTED BY VESSEL

Mark No.	Part of bag.	Date of sampling.	Polarization.	Loss in polarization.	Moisture.	Reducing sugar.	Gain in reducing sugar.	Ash.	Moisture ratio.	Deterioration predicted from moisture ratio.	Number of microorganisms per gram.	Deterioration predicted from number of microorganisms.	Molds.
					Per cent.	*Per cent.*	*Per cent.*	*Per cent.*					*Per cent.*
F 1....	Middle.	Mar. 18	93.2	.....	2.90	1.83	.....	0.30	0.43	+	35,000	+	1
		Apr. 15	92.5	0.7	2.80	1.99	0.16	.....	.37	+	1,650,000	+	*
		May 13	91.4	1.8	3.13	3.13	1.30	.....	.37	....	30,000	.....	*
	Surface.	Mar. 18	94.2	.....	2.31	1.30	.....	.30	.40	+	8,000	+	11
		Apr. 15	92.7	1.5	2.34	2.10	.80	.....	.32	+	18,000	+	3
		May 13	94.8	.....	1.70	1.26	.....	.....	.33	....	38,000	.....	*
F 2....	Middle.	Mar. 18	98.2	.....	.80	.54	.....	.28	.44	+	9,000	+	2
		Apr. 15	97.5	.7	.96	.59	.05	.....	.38	+	170,000	+	*
		May 13	96.9	1.3	1.00	.77	.23	.....	.32	....	17,000	.....	2
	Surface.	Mar. 18	97.9	.....	.75	.58	.....	.27	.36	+	2,000	+	10
		Apr. 15	98.4	.....	.46	.50	.....	.....	.29	−	7,600	+	0
		May 13	97.8	.1	.70	.46	.....	.....	.31	....	2,100	.....	0
F 3....	Middle.	Mar. 18	96.4	.....	1.70	.63	.....	.33	.47	+	134,000	+	0
		Apr. 15	95.8	.6	1.62	.96	.33	.....	.39	+	7,000,000	+	0
		May 13	95.8	.6	1.67	1.50	.87	.....	.40	....	10,000	.....	*
	Surface.	Mar. 18	97.0	.....	1.20	.66	.....	.36	.40	+	156,000	+	0
		Apr. 15	96.6	.4	1.27	.85	.19	.....	.37	+	5,500,000	+	0
		May 13	95.8	1.2	1.50	1.37	.71	.....	.36	....	30,000	.....	0
Port 1.	Middle.	Mar. 18	96.5	.....	1.08	1.06	.....	.39	.31	+	240	+	3
		Apr. 15	96.3	.2	1.00	1.05	.....	.....	.27	−	1,500	+	3
		May 13	95.9	.6	1.30	.93	.....	.....	.31	....	6,000	.....	1
	Surface.	Mar. 18	96.5	.....	1.10	1.08	.....	.42	.31	+	300	+	0
		Apr. 15	96.5	0	1.02	.98	.....	.....	.29	−	800	+	0
		May 13	96.3	.2	1.10	1.04	.....	.....	.30	....	7,000	.....	0
Port 2.	Middle.	Mar. 18	96.0	.....	1.18	1.16	.....	.45	.30	+	6,000	+	0
		Apr. 15	95.9	.1	1.10	1.09	.....	.....	.27	−	98	−	2
		May 13	95.8	.2	1.28	.97	.....	.....	.30	....	2,250	.....	0
	Surface.	Mar. 18	96.2	.....	1.16	1.30	.....	.42	.30	+	1,150	+	0
		Apr. 15	96.0	.2	1.10	1.21	.....	.....	.28	−	150	−	0
		May 13	95.8	.4	1.19	1.02	.....	.....	.30	....	8,000	.....	*
Port 3.	Middle.	Mar. 18	95.5	.....	1.42	1.17	.....	.51	.32	+	1,000	+	10
		Apr. 15	95.5	0	1.25	1.09	.....	.....	.28	−	2,100	+	0
		May 13	95.1	.4	1.42	1.04	.....	.....	.30	....	1,800	.....	1
	Surface.	Mar. 18	95.4	.....	1.42	1.04	.....	.55	.31	+	3,100	+	0
		Apr. 15	95.5	.....	1.10	1.05	.01	.....	.24	−	22,000	+	0
		May 13	95.4	0	1.37	.98	.....	.....	.30	....	2,150	.....	0

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[2] * Indicates negligible amount of mold.

TABLE I.—*Chemical and bacteriological analyses of Cuban raw sugars in storage*—Con.

TRANSPORTED BY VESSEL—continued

Mark No.	Part of bag.	Date of sampling.	Polarization.	Loss in polarization.	Moisture.	Reducing sugar.	Gain in reducing sugar.	Ash.	Moisture ratio.	Deterioration predicted from moisture ratio.	Number of microorganisms per gram.	Deterioration predicted from number of microorganisms.	Molds.
					Per cent.	*Per cent.*	*Per cent.*	*Per cent.*					*Per cent.*
Cun 1..	Middle.	Mar. 22	94.4	.....	2.00	0.98	.....	1.02	0.36	+	200	+	0
		Apr. 19	95.4	.....	1.75	.76	.....	.....	.31	+	400	+	0
		May 17	95.8	.....	1.71	.47	.....	.....	.40	....	8,000	.....	0
	Surface.	Mar. 22	94.2	.....	1.94	1.10	.....	.....	.34	+	400	+	0
		Apr. 19	94.4	.....	2.10	.90	.....	.....	.46	+	85	−	0
		May 17	94.4	.....	2.15	.75	.....	.....	.38	....	1,100	.....	0
Cun 2..	Middle.	Mar. 22	94.5	.....	1.68	1.19	.....	.96	.30	+	1,700	+	1
		Apr. 19	94.1	.4	2.10	1.24	.05	.....	.36	+	110,000	+	0
		May 17	93.5	1.0	2.21	1.33	.14	.....	.34	....	120,000	.....	0
	Surface.	Mar. 22	94.4	.....	1.50	1.24	.....	.99	.27	−	1,350	+	0
		Apr. 19	94.5	.....	1.85	1.17	.....	.....	.34	+	2,500	+	0
		May 17	92.0	.....	2.00	1.91	.67	.....	.25	....	160,000	.....	0
Cun 3..	Middle.	Mar. 22	95.3	.....	1.27	.90	.....	.81	.27	−	575	+	77
		Apr. 19	95.3	0	1.52	.81	.....	.....	.32	+	190	−	17
		May 17	95.0	.....	1.70	.83	.....	.....	.24	....	5,000	.....	1
	Surface.	Mar. 22	95.8	.....	1.30	.87	.....	.80	.31	+	350	+	94
		Apr. 19	95.0	.8	1.74	.85	.....	.....	.35	+	190	−	71
		May 17	94.8	1.0	1.66	.77	.....	.....	.32	....	480	.....	7
Cun 4..	Middle.	Mar. 22	97.0	.....	1.32	.52	.....	.50	.44	+	14,000	+	0
		Apr. 19	95.3	1.7	1.80	1.35	.83	.....	.38	+	100,000	+	0
		May 17	93.8	3.2	2.08	2.14	1.62	.....	.33	....	19,000	.....	*
	Surface.	Mar. 22	97.5	.....	1.04	.50	.....	.48	.35	+	14,000	+	0
		Apr. 19	96.2	.8	1.51	.51	.01	.....	.40	+	20,000	+	0
		May 17	94.0	3.0	1.81	2.13	1.63	.....	.30	....	4,100	.....	0
Cun 5..	Middle.	Mar. 22	93.8	.....	2.21	1.01	.....	1.02	.36	+	600	+	25
		Apr. 19	94.0	.....	2.33	.92	.....	.....	.39	+	2,700	+	2
		May 17	94.5	.....	2.34	.51	.....	.....	43	....	22,000	.....	*
	Surface.	Mar. 22	94.2	.....	2.10	1.01	.....	.98	.36	+	235	+	4
		Apr. 19	94.8	.....	1.83	.61	.....	.....	.35	+	336	+	4
		May 17	94.6	.....	1.89	.64	.....	.....	.35	....	1,000	.....	2
Cun 6..	Middle.	Mar. 22	96.2	.....	1.03	.99	.....	.72	.27	−	125	−	0
		Apr. 19	95.4	.8	1.53	1.01	.02	.....	.33	+	250	+	3
		May 17	94.9	1.3	1.70	1.08	.09	.....	.33	....	3,000	.....	3
	Surface.	Mar. 22	95.5	.....	1.20	1.22	.....	.78	.27	−	475	+	10
		Apr. 19	94.9	.6	1.80	1.16	.....	.....	.35	+	575	+	3
		May 17	95.2	.....	1.46	1.06	.....	.....	.30	....	560	.....	8
Agr 1..	Middle.	Mar. 30	96.6	.....	1.02	.89	.....	.48	.30	+	67	−	0
		Apr. 27	96.3	.3	1.20	.82	.....	.....	.33	+	1,000	+	25
		May 21	96.0	.6	1.30	.84	.....	.....	33	....	3,100	.....	3
	Surface.	Mar. 30	96.7	.....	1.00	.83	.....	.50	.30	+	125	−	0
		Apr. 27	96.3	.4	1.16	.88	.05	.....	.31	+	350	+	5
		May 21	96.2	.5	1.04	.80	.....	.....	.27	....	930	.....	0
Agr 2..	Middle.	Mar. 30	97.3	.....	.94	.57	.....	.48	.35		120,000		0
		Apr. 27	96.8	.5	1.16	.77	.20	.....	.36		115,000		*
		May 21	96.0	1.3	1.08	1.04	.47	.....	.27	....	145,000		□
	Surface.	Mar. 30	97.4	.....	.94	.58	.....	.48	.36		18,000		1
		Apr. 27	97.5	.....	.90	.45	.....	.....	.36		7,500		0
		May 21	97.3	.1	.90	.53	.08	.....	.30		215		□
Agr 3..	Middle.	Mar. 30	96.6	.....	1.02	.78	.....	.54	.30		190		*
		Apr. 27	96.4	.2	1.10	.76	.....	.....	.30		1,800		3
		May 27	96.3	.3	1.00	.77	.....	.....	.33		1,150		0
	Surface.	Mar. 30	96.5	...	1.07	1.60	.....	.59	.30		283		10
		Apr. 27	96.4	.1	1.09	.81	.....	.....	.30		300		28
		May 21	96.5	.....	1.14	.81	.....	.....	.33		220		0
Cab 1..	Middle.	Mar. 30	94.8	.....	1.30	1.45	.....	.57	.25		300		0
		Apr. 27	94.7	.1	1.50	1.48	.03	.....	.28		55,750		*
		May 21	95.6	.....	1.59	.80	.....	.....	.36		225,000		0
	Surface.	Mar. 30	94.2	.....	1.70	1.60	.....	.59	.29		250		2
		Apr. 27	94.4	.....	1.62	1.53	.....	.....	.29		2,200		*
		May 21	94.8	.....	1.60	1.04	.....	.....	.31		55,000		0
Cab 2..	Middle.	Mar. 30	94.8	.....	1.28	1.44	.....	.57	.25		290		0
		Apr. 27	94.8	.....	1.45	1.37	.....	.....	.27		165		0
		May 21	95.0	.....	1.54	1.35	.....	.....	.31		2,000		□
	Surface.	Mar. 30	94.7	.....	1.40	1.42	.....	.52	.26		210		*
		Apr. 27	94.6	.1	1.31	1.42	.....	.....	.24		500		3
		May 21	94.5	.2	1.43	1.42	.....	.....	.26		350		

TABLE I.—*Chemical and bacteriological analyses of Cuban raw sugars in storage*—Con.

TRANSPORTED BY VESSEL—continued

Mark No.	Part of bag.	Date of sampling.	Polarization.	Loss in polarization.	Moisture.	Reducing sugar.	Gain in reducing sugar.	Ash.	Moisture ratio.	Deterioration predicted from moisture ratio.	Number of microorganisms per gram.	Deterioration predicted from number of microorganisms.	Molds.
					Per cent.	*Per cent.*	*Per cent.*	*Per cent.*					*Per cent.*
Cab 3..	Middle.	Mar. 30	95.2	.....	1.80	1.02	.....	0.61	0.38		110,000		0
		Apr. 27	95.3	.....	1.86	.80	.....	.....	.40		6,000,000		0
		May 21	95.8	.....	1.64	.76	.....	.....	.40		52,000		0
	Surface.	Mar 30	94.8	.....	1.74	1.35	.....	.69	.33		1,260		1
		Apr. 27	95.4	.....	1.30	.87	.....	.....	.28		110,000		0
		May 21	95.4	.....	1.33	.69	.....	.....	.29		8,000		0

TRANSPORTED BY RAILWAY

Am 1..	Middle.	Apr. 1	92.0	.....	4.00	1.14	.....	0.40	0.50		4,000		1
		Apr. 29	90.8	1.2	3.40	2.24	1.10	.....	.37		120,000		1
		May 24	89.5	2.5	3.21	3.44	2.30	.....	.31		33,000		*
	Surface.	Apr. 1	96.0	.....	1.01	.81	.....	.48	.25		2,400		17
		Apr. 29	94.8	1.2	1.50	1.62	.81	.....	.30		4,900		0
		May 24	92.5	3.5	2.00	2.74	1.98	.....	.27		1,900		0
Am 2..	Middle.	Apr. 1	95.4	.....	1.60	1.47	.....	.36	.35		19,000		0
		Apr. 20	94.2	1.2	1.35	2.34	.87	.....	.23		90,000		0
		May 24	93.0	2.4	1.96	3.01	.....	.....	.28		20.000		*
	Surface.	Apr. 1	96.8	.....	.94	.76	.....	.33	.30		850		3
		Apr. 29	97.0	.....	.90	.91	.15	.....	.30		14,000		0
		May 24	95.7	1.1	1.15	1.54	.78	.....	.27		1,230		13
Am 3..	Middle.	Apr. 1	95.5	.....	1.92	.98	.....	.36	.43		30,000		*
		May 29	95.0	.5	1.30	2.00	1.02	.....	.26		65,000		1
		May 24	94.5	1.0	1.41	2.29	1.31	.....	.26		2,100		3
	Surface.	Apr. 1	96.8	.....	.90	.77	.....	.33	.29		4,000		1
		Apr. 29	96.5	.3	1.00	1.12	.35	.....	.29		4,000		*
		May 24	95.0	1.8	1.43	2.15	1.38	.....	.29		400		0
Am 4..	Middle.	Apr. 1	95.8	.....	1.76	.81	.....	.45	.42		50,000		*
		Apr. 29	94.4	1.4	1.47	1.69	.88	.....	.26		3,000		*
		May 24	93.5	2.3	1.80	2.19	1.38	.....	.28		750		0
	Surface.	Apr. 1	96.2	.....	1.12	.91	.05	.45	.30		1,500		2
		Apr. 29	96.2	.....	1.14	.96	.....	.....	.30		280		0
		May 24	95.8	.4	1.10	1.26	.35	.....	.26		540		8
Am 5..	Middle.	Apr. 1	96.8	.....	1.32	.69	.....	.36	.41		7,000		*
		Apr. 29	95.2	1.6	1.16	1.92	1.23	.....	.24		115,000		*
		May 24	94.8	2.0	1.33	2.29	1.60	.....	.24		2.000		0
	Surface.	Apr. 1	97.2	.....	.90	.70	.....	.39	.32		340		20
		Apr. 29	96.5	.7	.94	.98	.28	.....	.27		40.000		1
		May 24	97.0	.....	.85	.78	.08	.....	.28		1,420		5
Am 6..	Middle.	Apr. 1	95.5	.....	2.00	1.04	.....	.39	.44		300.000		*
		Apr. 29	95.7	.....	1.09	1.71	.67	.....	.25		120,000		*
		May 24	94.3	1.2	1.32	2.26	1.22	.....	.23		16,000		0
	Surface.	Apr. 1	97.0	.....	1.24	.73	.....	.40	.41		300		8
		Apr. 29	97.2	.....	1.02	.84	.11	.....	.36		10,000		0
		May 24	97.4	.....	.80	.76	.....	.....	.31		450		0
O 1	Middle.	Apr. 5	96.6	.....	80	.66	.....	.54	.24	—	1,700	+	3
		May 3	96.3	.3	.76	.75	.09	.....	.21	—	435	+	0
		May 26	96.4	.2	.85	.75	.09	.....	.24		415		0
	Surface.	Apr. 5	96.9	.....	.59	.75	.....	.55	.19	—	335	+	1
		May 3	96.6	.3	.86	.76	.01	.....	.25	—	200	+	17
		May 26	96.9	.....	.76	.75	.....	.....	.21	—	350		15
O 2	Middle.	Apr. 5	96.9	.....	.68	.64	.....	.57	.22	—	550	*	10
		May 3	96.6	.3	.70	.62	.02	.....	.21	—	140	—	0
		May 26	96.8	.1	.60	.64	.....	.....	.19		2,100		0
	Surface.	Apr. 5	97.0	.....	.60	.70	.....	.57	.20	—	275	+	2
		May 3	96.2	.8	1.00	.68	.....	.....	.26	—	500	*	*
		May 26	96.5	.5	.93	.69	.....	.....	.27	—	4,500		*
O 3	Middle.	Apr. 5	96.3	.....	.95	.75	.....	.63	.26	—	700	+	*
		May 3	95.9	.4	1.00	.77	.02	.....	.24	—	1,500	+	0
		May 26	96.1	.2	.93	.90	.15	.....	.24		1,100		0
	Surface.	Apr. 5	96.5	.....	.76	.75	.....	.63	.18	—	630	+	5
		May 3	95.9	.6	1.00	.83	.08	.....	.24	—	2,000	+	2
		May 26	96.2	.3	.90	.87	.12	.....	.24		1,300		0

TABLE I.—*Chemical and bacteriological analyses of Cuban raw sugars in storage*—Continued

TRANSPORTED BY RAILWAY—continued

Mark No.	Part of bag.	Date of sampling.	Polarization.	Loss in polarization.	Moisture.	Reducing sugar.	Gain in reducing sugar.	Ash.	Moisture ratio.	Deterioration predicted from moisture ratio.	Number of microorganisms per gram.	Deterioration predicted from number of microorganisms.	Molds.
					Per cent.	*Per cent.*	*Per cent.*	*Per cent.*					*Per cent.*
Pil 1	Middle.	Apr. 5	96.0	.....	1.36	0.52	.....	0.42	0.34	+	900	+	2
		May 3	95.9	.1	1.20	.60	.08	.....	.29	—	245,000	+	*
		May 26	96.6	.....	1.42	.45	.....	.....	.42		53,000		1
	Surface.	Apr. 5	95.7	.....	1.10	1.05	.....	.45	.26	—	340	+	4
		May 3	96.4	.....	1.02	.47	.....	.....	.28	—	240,000	+	*
		May 26	96.7	.....	.81	.55	.....	.....	.25		39,000		0
Pil 2	Middle.	Apr. 5	95.6	.....	1.70	.70	.....	.51	.39	+	4,250	+	0
		May 3	95.2	.4	1.53	.75	.05	.....	.32	+	80,000	+	0
		May 26	95.0	.6	1.20	1.26	.56	.....	.24		26,000		0
	Surface.	Apr 5	95.8	.....	1.30	1.10	.....	.54	.31	+	550	*	1
		May 3	95.9	.....	1.01	.49	.....	.....	.25	—	.....		0
		May 26	95.8	.....	1.06	.92	.....	.....	.25		1,850	+	.....
Pil 2	Middle.	Apr. 5	94.4	.....	1.88	1.35	.....	.42	.34	+	210,000	+	0
		May 3	94.4	.....	1.60	1.36	.01	.....	.29	—	210,000	+	*
		May 26	94.0	.4	1.54	1.74	.39	.....	.26		5,500		0
	Surface.	Apr. 5	96.5	.....	1.00	.94	.....	.40	.29	—	4,750	+	0
		May 3	95.4	1.1	1.70	.78	.....	.....	.37	+	26,000	+	*
		May 26	95.6	.9	1.35	1.32	.38	.....	.31		1,900		9

TRANSPORTED BY RAILWAY AND VESSEL

M 1	Middle.	Apr. 5	96.0	.....	1.10	0.61	.....	0.70	0.28	—	120,000	+	0
		May 3	95.3	0.7	1.30	.77	0.16	.....	.28	—	75,000	+	*
		May 26	95.4	.6	1.17	1.20	.59	.....	.25		130,000		*
	Surface.	Apr. 5	96.5	.....	.70	.68	.....	.66	.20	—	2,300	+	4
		May 3	95.6	.9	1.29	.72	.04	.....	.29	—	Lost		0
		May 26	94.8	1.7	1.36	1.34	.62	.....	.26		360		5
M 2	Middle.	Apr. 5	96.2	.....	.86	1.05	.....	.48	.23	—	1,050	+	2
		May 3	95.9	.3	1.15	1.04	.....	.....	.28	—	70	—	0
		May 26	96.2	.....	1.02	1.04	.....	.....	.27		400		0
	Surface.	Apr. 5	97.0	.....	.62	.97	.....	.50	.27	—	260	+	0
		May 3	96.0	1.0	1.23	.97	.....	.....	.31	+	370	+	0
		May 26	96.4	.6	.85	.93	.....	.....	.23		225		0
M 3	Middle.	Apr. 5	96.3	.....	1.16	.48	.....	.66	.31	+	350	+	0
		May 3	95.8	.5	1.27	.83	.35	.....	.30	*	100,000	+	*
		May 26	95.2	1.1	1.33	1.53	1.05	.....	.28		200,000		*
	Surface.	Apr. 5	96.3	.....	.90	.75	.....	.65	.24	—	800,000	+	0
		May 3	96.0	.3	1.05	.83	.08	.....	.26	—	550	+	*
		May 26	96.2	.1	1.03	.83	.08	.....	.27		27,000		0
Ag 1	Middle.	Apr. 5	96.0	.....	1.00	1.04	.....	.63	.25	—	175	—	0
		May 3	95.5	.5	1.16	1.08	.04	.....	.26	—	250	+	0
		May 26	95.8	.2	1.04	.90	.....	.....	.21		650		8
	Surface.	Apr. 5	96.3	.....	.92	1.16	.....	.60	.25	—	310	+	0
		May 3	95.6	.7	1.26	1.01	.....	.....	.29	—	2,600	+	*
		May 26	95.9	.4	1.29	1.06	.....	.....	.31		540		0
Ag 2	Middle.	Apr. 5	96.6	.....	.75	.85	.....	.54	.22	—	325	+	0
		May 3	95.6	1.0	1.20	.98	.13	.....	.27	—	4,000	+	0
		May 26	96.0	.6	1.03	.95	.10	.....	.26		4,500		*
	Surface.	Apr. 5	96.8	.....	.70	.93	.....	.54	.22	—	200	+	30
		May 3	96.3	.5	1.08	.87	.....	.....	.29	—	4,500	+	2
		May 26	96.5	.3	.88	.90	.....	.....	.25		150		0
Ag 3	Middle.	Apr. 5	94.4	.....	1.35	1.28	.....	.80	.24	—	117	—	9
		May 3	94.2	.2	1.38	1.50	.22	.....	.24	—	200	+	5
		May 26	94.2	.2	1.56	1.47	.19	.....	.27		2,750		1
	Surface.	Apr. 5	94.8	.....	1.16	1.41	.....	.78	.22	—	230	+	33
		May 3	93.8	1.0	1.63	1.42	.01	.....	.31	+	450	+	0
		May 26	94.2	.6	1.43	1.39	.....	.....	.25		1,100		1

From the data given in Table I it will be seen that the sugars vary in initial polarization from 92 to 98.2; in moisture from 0.75 to 2.90; in percentage of reducing sugars from 0.52 to 1.83; in percentage of ash from

0.28 to 1.02; in moisture ratio from 0.18 to 0.50; in number of microorganisms per gram from 67 to 134,000; and in percentage of molds from 0 to 94. It is apparent that certain generalizations may be drawn from Table I—namely, that there is a reduction in polarization in practically all sugars during storage, a fact already established. Furthermore, it is apparent that a decrease in polarization is generally accompanied by an increase in reducing sugars. As might be anticipated, when deterioration sets in during the first four weeks of incubation, it continues through the second four weeks, although it would be difficult to state whether the deterioration is more active in the second period of four weeks than the first. While it is not to be expected that the number of microorganisms present can be correlated with polarization, nevertheless, in general, the greatest number of microorganisms occurs where the moisture ratio is highest, and as a corollary we have observed that the lighter colored sugars having the higher moisture ratios deteriorate most rapidly.

The temperature and relative humidity in New Orleans during the months of storage of these sugars are given in Table II. It may be said that in 1920 these were somewhat lower than the average. Table III graphically represents the differences between successive samplings together with a comparison between the last sampling and the first. There is a fairly close agreement to be found between the results for bags of one mark; therefore these bags have been summarized in Table IV.

TABLE II.—*Temperature and relative humidity at New Orleans, La., during March, April, and May, 1920*

Month.	Relative humidity.	Temperature.		
		Maximum.	Minimum.	Mean.
		°F.	°F.	°F.
March	88	84	27	59.40
April	80	87	39	65.85
May	75	92	57	75.75
Average	81	88	41	67.00

TABLE III.—*Differences between successive samplings of sugars in normal storage*[1]

Mark No.	Part of bag.	Date of sampling.	Polarization.	Moisture.	Reducing sugar.	Moisture ratio.	Number of microorganisms per gram.	Molds.
				Per cent.	*Per cent.*			
F 1	Middle	Apr. 15	−	−	+	−	+	−
		May. 13	−	+	+	*	−	*
		Mar. 18	−	+	+	−	−	−
	Surface	Apr. 15	−	+	+	−	+	−
		May 13	+	−	−	+	+	−
		[2]Mar. 18	+	−	−	−	+	−

[1] * signifies no change; + signifies increase; − signifies decrease.
[2] Third sampling compared with first.

TABLE III.—*Differences between successive samplings of sugars in normal storage*—Continued

Mark No.	Part of bag.	Date of sampling.	Polarization.	Moisture.	Reducing sugar.	Moisture ratio.	Number of microorganisms per gram.	Molds.
				Per cent.	*Per cent.*			
F 2.....	Middle.	Apr. 15	−	+	+	−	+	−
		May 13	−	+	+	−	−	+
		[2]Mar. 18	−	+	+	−	+	*
	Surface.	Apr. 15	+	−	−	−	+	−
		May 13	−	+	−	+	−	*
		[2]Mar. 18	−	−	−	−	+	*
F 3.....	Middle.	Apr. 15	−	−	+	−	+	*
		May 13	*	+	+	+	−	+
		[2]Mar. 18	−	−	+	−	−	+
	Surface.	Apr. 15	−	+	+	−	+	*
		May 13	−	+	+	−	−	*
		[2]Mar. 18	−	+	+	−	−	*
Port 1....	Middle.	Apr. 15	−	−	−	−	+	*
		May 13	−	+	−	+	+	−
		[2]Mar. 18	−	+	−	*	+	−
	Surface.	Apr. 15	*	−	−	−	+	*
		May 13	−	+	+	+	+	*
		[2]Mar. 18	−	*	−	−	+	*
Port 2....	Middle.	Apr. 15	−	−	−	−	−	+
		May 13	−	+	−	+	+	−
		[2]Mar. 18	−	+	−	*	−	*
	Surface.	Apr. 15	−	−	−	−	−	*
		May 13	−	+	−	+	+	+
		[2]Mar. 18	−	+	−	*	+	+
Port 3....	Middle.	Apr. 15	*	−	−	−	+	−
		May 13	−	+	−	+	−	+
		[2]Mar. 18	−	*	−	−	+	−
	Surface.	Apr. 15	+	−	+	−	+	*
		May 13	−	+	−	−	−	*
		[2]Mar. 18	*	−	−	−	−	*
Cun 1....	Middle.	Apr. 19	+	−	−	−	*	*
		May 17	+	−	−	+	+	*
		[2]Mar. 22	+	−	−	+	+	*
	Surface.	Apr. 19	+	+	−	+	−	*
		May 17	*	+	−	−	+	*
		[2]Mar. 22	+	+	−	+	+	*
Cun 2....	Middle.	Apr. 19	−	+	+	+	+	−
		May 17	−	+	+	−	+	*
		[2]Mar. 22	−	+	+	+	+	−
	Surface.	Apr. 19	+	+	−	+	+	*
		May 17	−	+	+	−	+	*
		[2]Mar. 22	−	+	+	−	+	*
Cun 3....	Middle.	Apr. 19	*	+	−	+	−	−
		May 17	−	+	+	+	−	−
		[2] Mar. 22	−	+	−	−	+	−
	Surface.	Apr. 19	−	+	−	+	−	−
		May 17	−	−	−	−	+	−
		[2] Mar. 22	−	+	−	+	+	−
Cun 4....	Middle.	Apr. 19	−	+	+	−	+	*
		May 17	−	+	+	−	−	*
		[2] Mar. 22	−	+	+	−	+	+
	Surface.	Apr. 19	−	+	+	+	+	*
		May 17	−	+	+	−	−	*
		[2] Mar. 22	−	+	+	−	−	*

[2] Third sampling compared with first.

TABLE III.—*Differences between successive samplings of sugars in normal storage*—Continued

Mark No.	Part of bag.	Date of sampling.	Polarization.	Moisture.	Reducing sugar.	Moisture ratio.	Number of microorganisms per gram.	Molds.
				Per cent.	*Per cent.*			
Cun 5	Middle	Apr. 19	+	+	−	+	+	−
		May 17	+	+	−	+	+	−
		[2] Mar. 22	+	+	−	+	+	−
	Surface	Apr. 19	+	−	−	−	+	*
		May 17	−	+	+	*	+	−
		[2] Mar. 22	+	−	−	−	+	−
Cun 6	Middle	Apr. 19	−	+	+	+	+	+
		May 17	−	+	+	*	+	*
		[2] Mar. 22	−	+	+	+	+	+
	Surface	Apr. 19	−	+	−	+	+	−
		May 17	+	−	−	−	−	+
		[2] Mar. 22	−	+	−	+	+	−
Agr 1	Middle	Apr. 27	−	+	−	+	+	+
		May 21	−	+	+	*	+	−
		[2] Mar. 30	−	+	−	+	+	+
	Surface	Apr. 27	−	+	+	+	+	+
		May 21	−	−	−	−	+	−
		[2] Mar. 30	−	+	−	−	+	*
Agr 2	Middle	Apr. 27	−	+	+	+	−	*
		May 21	−	−	+	−	+	+
		[2] Mar. 30	−	+	+	−	+	+
	Surface	Apr. 27	+	−	−	*	−	+
		May 21	−	*	+	−	−	−
		[2] Mar. 30	−	−	−	−	−	*
Agr 3	Middle	Apr. 27	−	+	−	*	+	+
		May 21	−	−	+	+	−	+
		[2] Mar. 30	−	−	−	+	+	+
	Surface	Apr. 27	−	+	−	*	+	+
		May 21	+	+	*	+	−	+
		[2] Mar. 30	*	+	−	+	−	+
Cab 1	Middle	Apr. 27	−	+	+	+	+	*
		May 21	+	+	−	+	+	+
		[2] Mar. 30	+	+	−	+	+	+
	Surface	Apr. 27	+	−	−	*	+	+
		May 21	+	−	−	+	+	−
		[2] Mar. 30	+	−	−	+	+	−
Cab 2	Middle	Apr. 27	*	+	−	+	−	*
		May 21	+	+	−	+	+	*
		[2] Mar. 30	+	+	−	+	+	*
	Surface	Apr. 27	−	−	*	−	+	+
		May 21	−	+	*	+	−	+
		[2] Mar. 30	−	+	*	*	+	+
Cab 3	Middle	Apr. 27	+	+	−	+	*	*
		May 21	+	−	−	*	−	*
		[2] Mar. 30	+	−	−	+	−	*
	Surface	Apr. 27	+	−	−	+	+	−
		May 21	*	+	−	+	−	*
		[2] Mar. 30	+	−	−	−	+	−
Am 1	Middle	Apr. 29	−	−	+	−	+	*
		May 24	−	−	+	−	−	−
		[2] Apr. 1	−	−	+	−	+	−
	Surface	Apr. 29	−	+	+	+	+	−
		May 24	−	+	+	−	−	*
		[2] Apr. 1	−	+	+	+	−	−

[2] Third sampling compared with first.

TABLE III.—*Differences between successive samplings of sugars in normal storage*—Continued

Mark No.	Part of bag.	Date of sampling.	Polarization.	Moisture.	Reducing sugar.	Moisture ratio.	Number of microorganisms per gram.	Molds.
				Per cent.	*Per cent.*			
Am 2	Middle	Apr. 29	−	−	+	−	+	*
		May 24	−	+	+	+	−	+
		[2] Apr. 1	−	+	+	−	+	+
	Surface	May 29	+	−	+	*	+	−
		May 24	−	+	+	−	−	+
		[2] Apr. 1	−	+	+	−	+	+
Am 3	Middle	Apr. 29	−	−	+	−	+	+
		May 24	−	+	+	*	−	+
		[2] Apr. 1	−	−	+	−	−	+
	Surface	Apr. 29	−	+	+	*	*	−
		May 24	−	+	+	*	−	−
		[2] Apr. 1	−	+	+	*	−	−
Am 4	Middle	Apr. 29	−	−	+	−	−	*
		May 24	−	+	+	+	−	−
		[2] Apr. 1	−	+	+	−	−	−
	Surface	Apr. 29	*	+	+	*	−	−
		May 24	−	−	+	−	+	+
		[2] Apr. 1	−	−	+	−	−	+
Am 5	Middle	Apr. 29	−	−	+	−	+	*
		May 24	−	+	+	*	−	−
		[2] Apr. 1	−	+	+	−	−	−
	Surface	Apr. 29	−	+	+	−	+	−
		May 24	+	−	−	+	−	+
		[2] Apr. 1	−	−	+	−	+	−
Am 6	Middle	Apr. 29	+	−	+	−	−	*
		May 24	−	+	+	−	−	−
		[2] Apr. 1	−	−	+	−	−	−
	Surface	Apr. 29	+	−	+	−	+	−
		May 24	+	−	−	−	−	*
		[2] Apr. 1	+	−	+	−	+	−
O 1	Middle	May 3	−	−	+	−	−	−
		May 26	+	+	*	+	−	*
		[2] Apr. 5	−	−	+	*	−	−
	Surface	May 3	−	+	+	+	−	+
		May 26	+	−	−	−	+	−
		[2] Apr. 5	*	+	*	+	+	+
O 2	Middle	May 3	−	+	−	−	−	−
		May 26	+	−	+	−	+	*
		[2] Apr. 5	−	−	*	−	+	*
	Surface	May 3	−	+	−	+	+	−
		May 26	+	−	+	+	+	*
		[2] Apr. 5	−	+	−	+	+	−
O 3	Middle	May 3	−	+	+	−	+	−
		May 26	+	−	−	*	−	*
		[2] Apr. 5	−	−	+	−	+	−
	Surface	May 3	−	+	+	+	+	−
		May 26	+	−	+	*	−	−
		[1] Apr. 5	−	+	+	+	+	−
Pil 1	Middle	May 3	−	−	+	−	+	−
		May 26	+	+	−	+	−	+
		[2] Apr. 5	+	+	−	+	+	−
	Surface	May 3	+	−	−	+	+	−
		May 26	+	−	+	−	+	−
		[2] Apr. 5	+	−	−	−	+	−

[2] Third sampling compared with first.

TABLE III.—*Differences between successive samplings of sugars in normal storage*—Continued

Mark No.	Part of bag.	Date of sampling.	Polarization.	Moisture.	Reducing sugar.	Moisture ratio.	Number of microorganisms per gram.	Molds.
				Per cent.	*Per cent.*			
Pil 2	Middle	May 3	−	−	+	−	+	*
		May 26	−	−	+	−	−	*
		[2] Apr. 5	−	−	+	−	+	*
	Surface	May 3	+	−	−	−	.....	.....
		May 26	−	+	*	.....	.....	.....
		[2] Apr. 5	*	−	−	.....	.....	.....
Pil 3	Middle	May 3	*	−	+	−	*	+
		May 26	−	−	+	−	−	−
		[2] Apr. 5	−	−	+	−	−	*
	Surface	May 3	−	+	−	+	+	+
		May 26	+	−	+	−	−	+
		[2]Apr. 5	+	+	−	+	−	+
M 1	Middle	May 3	−	+	+	*	−	+
		May 26	+	−	+	−	+	*
		[2] Apr. 5	−	+	+	−	+	+
	Surface	May 3	−	+	+	+	.....	.....
		May 26	−	+	+	−	.....	.....
		[2] Apr. 5	−	+	+	+	.....	.....
M 2	Middle	May 3	−	+	−	+	−	−
		May 26	+	−	*	−	+	*
		[2] Apr. 5	*	+	−	+	−	−
	Surface	May 3	−	+	*	+	+	*
		May 26	+	−	−	−	−	*
		[2] Apr. 5	−	+	−	−	−	*
M 3	Middle	May 3	−	+	+	−	+	+
		May 26	−	+	+	−	+	*
		[2] Apr. 5	−	+	+	−	+	+
	Surface	May 3	−	+	+	+	−	+
		May 26	+	−	*	+	+	−
		[2] Apr. 5	−	+	+	+	−	*
Ag 1	Middle	May 3	−	+	+	+	+	*
		May 26	+	−	−	−	+	+
		[2] Apr. 5	−	+	−	−	+	+
	Surface	May 3	−	+	−	+	+	+
		May 26	+	+	+	+	+	−
		[2] Apr. 5	−	+	−	+	+	*
Ag 2	Middle	May 3	−	+	+	+	+	*
		May 26	+	−	−	−	+	+
		[2] Apr. 5	−	+	+	+	+	+
	Surface	May 3	−	+	−	+	+	−
		May 26	+	−	+	−	−	−
		[2] Apr. 5	−	+	−	+	−	−
Ag 3	Middle	May 3	−	+	+	*	+	−
		May 26	*	+	+	+	+	−
		[2] Apr. 5	−	+	+	+	+	−
	Surface	May 3	−	+	+	+	+	−
		May 26	+	−	−	−	+	+
		[2] Apr. 5	−	+	−	+	+	−

[2] Third sampling compared with first.

TABLE IV.—*Summary of differences between successive samplings (average of bags of same mark)*[1]

Mark No.	Part of bag.	Date of sampling.	Polarization.	Moisture.	Reducing sugar.	Moisture ratio.	Number of microorganisms per gram.	Molds.
				Per cent.	*Per cent.*			*Per cent.*
F 1 to 3	Middle	Apr. 15	−	−	+	−	+	−
		May 13	−	+	+	*	−	+
		[2]Mar. 18	−	+	+	−	−	*
	Surface	Apr. 15	−	+	+	−	+	−
		May 13	−	+	−	+	−	*
		[2]Mar. 18	−	−	+	−	+	*
Port 1 to 3	Middle	Apr. 15	−	−	−	−	+	*
		May 13	−	+	−	+	+	−
		[2]Mar. 18	−	+	−	+	+	−
	Surface	Apr. 15	*	−	−	−	+	*
		May 13	−	+	−	+	+	*
		[2]Mar. 18	−	*	−	−	+	*
Cun 1 to 6	Middle	Apr. 9	−	+	*	+	+	−
		May 17	−	+	+	+	+	−
		[2]Mar. 22	−	+	*	+	+	*
	Surface	Apr. 9	*	+	−	+	+	−
		May 17	−	+	*	−	+	*
		[2]Mar. 22	−	+	−	*	+	−
Agr 1 to 3	Middle	Apr. 27	−	+	−	+	+	+
		May 21	−	−	+	*	+	+
		[2]Mar. 30	−	+	−	+	+	+
	Surface	Apr. 9	−	+	−	*	+	+
		May 17	−	*	*	−	−	−
		[2]Mar. 22	−	+	−	−	−	*
Cab 1 to 3	Middle	Apr. 27	*	+	−	+	+	*
		May 21	+	+	−	+	+	+
		[2]Mar. 30	+	+	−	+	+	*
	Surface	Apr. 27	+	−	−	*	+	*
		May 21	*	+	−	+	−	*
		[2]Mar. 30	+	−	−	*	+	−
Am 1 to 6	Middle	Apr. 29	−	−	+	−	+	*
		May 24	−	+	+	*	−	−
		[2]Apr. 1	−	*	+	−	−	−
	Surface	Apr. 29	−	+	+	−	+	−
		May 24	−	*	+	−	−	+
		[2]Apr. 1	−	*	+	−	*	−
O 1 to 3	Middle	May 3	−	+	+	−	−	−
		May 26	+	−	*	*	−	*
		[2]Apr. 5	−	−	+	−	+	−
	Surface	May 3	−	+	+	+	+	*
		May 26	+	−	+	*	+	−
		[2]Apr. 5	−	+	*	+	+	−
Pil 1 to 3	Middle	May 3	−	−	+	−	+	*
		May 26	−	−	+	*	−	*
		[2]Apr. 5	−	−	+	−	+	*
	Surface	May 3	+	−	−	−	+	−
		May 26	+	−	+	*	*	−
		[2]Apr. 5	*	−	−	+	*	−
M 1 to 3	Middle	May 3	−	+	+	*	−	+
		May 26	+	−	+	−	+	*
		[2]Apr. 5	−	+	+	−	+	+
	Surface	May 3	−	+	+	+	*	+
		May 26	+	−	+	−	*	−
		[2]Apr. 5	−	+	+	+	−	*

[1] *signifies no change; +signifies increase, −signifies decrease.
[2] Third sampling compared with first.

TABLE IV.—*Summary of differences between successive samplings (average of bags of same mark)*—Continued

Mark No.	Part of bag.	Date of sampling.	Polarization.	Moisture.	Reducing sugar.	Moisture ratio.	Number of microorganisms per gram.	Molds.
				Per cent.	*Per cent.*			*Per cent.*
Ag 1 to 3.	Middle.	May 3	−	+	+	+	+	*
		May 26	+	−	−	−	+	+
		[2]Apr. 5	−	+	+	+	+	−
	Surface.	May 3	−	+	−	+	+	−
		May 26	+	−	+	−	+	−
		[2]Apr. 5	−	+	−	+	+	−

[2] Third sampling compared with first.

It will be seen that in practically all instances there has been a reduction in polarization between successive samplings. With regard to moisture content, however, there appears to be an increase in a majority of instances. It is interesting in this connection to note that, with the exception of the Cab sugars, an increase in polarization is accompanied by a decrease in moisture content. Naturally, this means that there has actually been a loss in weight of sugar. Furthermore, it will be seen that the surface of each bag decreased in moisture content, or dried out, as might be expected, much more rapidly than the middle of the same bag. In the sugars which have deteriorated it will be observed that there has been an increase in percentage of reducing sugars in successive samplings. However, as a rule this increase is more noticeable in the middle of the bag than at the surface where the deterioration does continue to progress at the initial rate. The conditions of temperature and humidity were such as to preclude the possibility of deterioration taking place more rapidly from the surface of the bag than from the interior of the bag, as occurs under average conditions which were noted in the previous experiment.[1] The moisture ratio was variable and does not permit of any generalization.

In considering the number of microorganisms it will be seen that in most instances there was an increase between successive samplings. In general it was found in corroboration of the results previously set forth that the increase in numbers of microorganisms was relatively more rapid during the first month of incubation than subsequently. Likewise it is to be noted that there is usually a greater number of microorganisms in the middle of the bag than at the surface, where drying out occurs. It will be shown in Table V, which is again corroborative of previous work, that there is correlation between the number of microorganisms and deterioration where the initial content is high or multiplication has been rapid. The percentage of molds is variable, and a tendency to decrease

[1] KOPELOFF, Nicholas, and PERKINS, H. Z. E. OP. CIT., 1920.

in the surface is to be noted during the first four weeks of incubation. It is evident, therefore, that these results agree very closely with those previously obtained, and this is of added significance when it is remembered that the range in variety of sugars is considerably greater.

TABLE V.—*Summary showing correlation between deterioration and number of microorganisms*

Mark No.	Part of bag.	Date of sampling.	Loss in polarization.	Gain in reducing sugar.	Number of microorganisms per gram.
				Per cent.	
Am 1	Middle	Third	2.5	2.30	120,000
	Surface	do	3.5	1.93	4,900
Cun 4	do	do	3.0	1.63	20,000
	Middle	do	3.2	1.58	100,000
Am 5	do	do	2.0	1.60	115,000
Am 4	do	do	2.3	1.38	50,000
Am 3	Surface	do	1.8	1.38	4,000
Am 3	Middle	do	1.0	1.31	65,000
F 1	do	do	1.8	1.30	1,650,000
Am 5	do	Second	1.6	1.23	70,000
Am 6	do	Third	1.2	1.22	120,000
Am 1	do	Second	1.2	1.10	4,000
M 3	do	Third	1.1	1.05	100,000
Am 3	do	Second	.5	1.02	30,000

Table V consists of a summary arranged in such a manner as to bring out clearly the correlation between the number of microorganisms and deterioration. The order of bags is based upon the increase in reducing sugars, since that represents the best criterion for determining deterioration. In addition, it will be noted that the loss in polarization is proportional to the gain in reducing sugars. Still more significant, however, is the fact that deterioration occurs in the presence of the maximum numbers of microorganisms. It may be mentioned that the number of microorganisms set down opposite any figure for gain in reducing sugars is the number occurring at the previous sampling, since that number was responsible for the deterioration found at the time of analysis. With three exceptions the greatest deterioration is to be found when there are more than 20,000 microorganisms per gram, and the average deterioration (represented by an increase of more than 1 per cent of reducing sugars) is to be found where there are 174,000 per gram. It is interesting to compare Table V with Table VI, which is a summary showing the maximum numbers of microorganisms where no deterioration has occurred. It will be seen at a glance that in only five instances has this number exceeded 8,000 per gram, the average being about 11,000 (unduly weighted because of the Cab sugar which was especially heavily infected). Thus, a comparison between Tables V and VI reveals quite clearly that large numbers of microorganisms are causally related to deterioration and that the converse is likewise true.

TABLE VI.—*Summary showing maximum numbers of microorganisms where no deterioration occurs*

Mark No.	Part of bag.	Number of microorganisms per gram.	Mark No.	Part of bag.	Number of microorganisms per gram.
P 1	Middle	6,000	Agr 1	Middle	3,100
	Surface	7,000	Agr 3	Middle	1,800
P 2	Middle	6,000		Surface	300
	Surface	8,000	Cab 2	Middle	2,000
P 3	Middle	2,100		Surface	500
	Surface	22,000	Cab 3	....do	110,000
C 1	Middle	8,000	M 3	Middle	1,050
	Surface	1,100		Surface	370
C 3	Middle	5,000	Ag 1	....do	2,600
	Surface	14,000	Ag 2	....do	4,500
C 6	Middle	22,000	Pil 1	....do	24,500
	Surface	1,000			

In Table VII the sugars analyzed have been ranked according to deterioration as based upon the greatest loss of polarization during normal storage. In compiling these data the analyses for all the bags of each mark were averaged. It is evident that the deterioration in the first six sugars mentioned was appreciable, the Am sugar being considerably more deteriorated than any others. Inasmuch as this sugar came by railroad as did the O and Pil sugars, it would be difficult to regard the means of transportation as the sole limiting factor. Since the former had a higher moisture ratio and considerably more microorganisms per gram, it is natural to suppose that it would deteriorate more rapidly under any environmental conditions.

TABLE VII.—*Sugars ranked according to greatest loss in polarization during normal storage*

Rank.	Mark.	Part of bag.	Average loss in polarization per bag.	Rank.	Mark.	Part of bag.	Average loss in polarization per bag.
1	Am	Middle	1.5	1	Am	Surface	0.8
2	F	...do	.9	2	M	...do	.8
3	Cun	...do	.7	3	Cun	...do	.7
4	Agr	...do	.6	4	Ag	...do	.6
5	M	...do	.5	5	F	...do	.5
6	Ag	...do	.4	6	O	...do	.4
7	Pil	...do	.3	7	Pil	...do	.3
8	O	...do	.2	8	Agr	...do	.2
9	Port	...do	.2	9	Port	...do	.1
10	Cab	...do	0	10	Cab	...do	0

It is interesting to note further in Table VII that in the majority of cases the rank of sugars with regard to deterioration is the same for the middle of the bag and for the surface. For example, the Am sugar shows greatest deterioration both in the middle and at the surface, while the Port and Cab sugars show least in both cases.

It has been shown that it is possible to predict the keeping quality of a sugar (from the standpoint of mold infection) by the simultaneous consideration of moisture ratio and number of organisms per gram.[1] Evidence for a prediction based on the number of bacteria was likewise advanced.[1] In Table I the plus and minus signs in the columns labeled "Deterioration predicted from moisture ratio" and "Deterioration predicted from number of microorganisms per gram" represent the prediction of deterioration based upon these factors considered independently. In this case we have taken the critical moisture ratio and the number of bacteria per gram which are required to produce deterioration in four weeks at this temperature and humidity of incubation as 30 and 200, respectively. (Table VIII.) Where these conditions were higher, as in the experiment of 1919,[2] less than half this number of microorganisms will produce similar effects. If attention is focused upon the moisture ratio it will be seen that the factor of safety as worked out by previous investigators holds true to a limited extent. In other words, where the moisture ratio is above 0.30 to 0.33 deterioration usually sets in, while sugars with lower moisture ratios usually resist deterioration. However, there are any number of instances where this factor of safety fails to function as an adequate criterion, and we may turn with some confidence to the number of microorganisms per gram as a true index of deterioration. In fact, a careful analysis of the data presented in Table I shows that as a criterion for predicting deterioration the moisture ratio or factor of safety proved to be in agreement with the analyses in 57 instances and failed in 86 instances; in other words, it was only 40 per cent effective. On the other hand, the use of the number of microorganisms per gram as an index of deterioration resulted in 96 successful predictions and 47 failures, or an efficiency of 67 per cent, which is 27 per cent better than the factor of safety. In the 65 cases where the moisture ratio is in agreement with number of microorganisms for the theoretical prediction of deterioration, there was practical confirmation in the majority of instances.

[1] KOPELOFF, Nicholas, and KOPELOFF, Lillian. OP. CIT., 1920.
[2] KOPELOFF, Nicholas, and PERKINS, H. Z. E. OP. CIT., 1920.

TABLE VIII.—*Correlation of moisture ratio with number of microorganisms* [1]

MOISTURE RATIO

	0.18 to 0.21.	0.22.	0.23.	0.24.	0.25.	0.26.	0.27.	0.28.	0.29.	0.30.	0.31.	0.32.	0.33.	0.34.	0.35.	0.36.	0.37.	0.38.	0.39.	0.40.	0.41.	0.42+.
0 to 100	...	...	...	*	*	...	*	−	...	...	...	...	...	...	...	...	...	...	...	...	...	−
100 to 200	*	+	...	*	*	...	*	+	...	*	...	+	...	...	...	−	+	...	...	...	...	...
200 to 300	+	...	...	...	−	*	+	...	−	*	+	...	+	...	...	−	−	...	...	...	−	...
300 to 400	*	+	...	...	*	−	...	...	...	−	*	+	...	...	...	...	−	...	...	...	...	...
400 to 500	*	...	*	...	...	+	*	...	...	...	...	...	+	−	...	...	−	...	...	...	...	...
500 to 1,000	...	...	...	...	...	...	*	...	−	*	...	−	...	...	...	−	...	...	...	...	...	+
1,000 to 5,000	+	...	...	*	...	+	...	−	+	+	−	...	−	+	...	−	...	...	*	...	...	...
5,000 to 10,000	...	...	...	...	...	...	...	...	−	...	...	...	...	...	...	*	+	...	...	+	...	+
10,000 to 25,000	...	...	...	−	...	...	...	...	...	...	...	−	...	...	+	−	...	...	...	+	...	+
25,000 to 50,000	...	...	...	...	...	...	−	*	...	...	...	...	...	...	...	+	...	...	...	...	...	+
50,000 to 100,000	...	...	+	...	...	+	...	*	...	...	...	+	...	...	...	+	...	*	...	...	...	...
100,000 to 250,000	...	...	...	+	+	...	...	+	...	...	...	...	...	−	+	+	+	+	...	...	+	+
250,000 to 500,000	...	...	...	...	...	...	...	−	*	...	...	...	...	...	...	−	...	...	...	+	...	−
500,000 to 1,000,000	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1,000,000 to 5,000,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...	+	...	...	...
5,000,000+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...

[1] + signifies deterioration; − signifies no deterioration; * signifies doubtful deterioration.

It will be seen from Table VIII, where there has been graphically illustrated the correlation between moisture ratio and number of microorganisms per gram that while such a relationship is of necessity dependent upon the environmental conditions at hand and it is hazardous in consequence to derive any didactic conclusions, nevertheless certain generalizations appear significant. For example, with more than 50,000 microorganisms per gram in practically all instances there was deterioration at every moitsure ratio employed. As the number of microorganisms is increased beyond this point it is almost certain that deterioration will occur at any moisture ratio generally occurring in Cuban raw sugar. As the number of organisms per gram is decreased to about 500 we have evidence of less deterioration at moisture ratios below 0.36. However, where the moisure ratio remains above 0.36 deterioration is effected by more than this number. On the other hand, even where the moisture ratio is reduced below 0.30, which is considered the critical point, there is ample evidence to indicate that deterioration may be induced by more than 200 microorganisms per gram. This corroborates the conclusions arrived at in the investigations previously referred to [1] and emphasizes again the necessity for reducing the mass infection in sugar. Thus, on the basis of polarization, moisture content, and bacteriological analysis, it is possible to predict the keeping quality of sugar and thereby introduce considerable economy by immediately disposing of those sugars which will deteriorate rapidly and storing only those proved to be capable of storage without serious loss. As a matter of actual manufacture, it should not be difficult to control the microorganisms to such an extent

[1] KOPELOFF, Nicholas, and PERKINS, H. Z. E., op. cit.
——— and KOPELOFF, LILLIAN, op. cit., 1919.
——— ——— op. cit., 1920.

as to inhibit their detrimental activities. In this connection it may be stated that recent experiments have enabled us to develop a method for eliminating the microorganisms in sugar by the use of superheated steam in the centrifugal which destroys over 90 per cent of the microorganisms.[1]

It is, therefore, evident that sugar deterioration depends upon the two factors of moisture ratio and number of microorganisms per gram. Furthermore, if the number of microorganisms is sufficiently reduced, and if the moisture ratio is properly controlled, sugar deterioration may be satisfactorily prevented.

SUMMARY

(1) From the results presented a correlation has been established between deterioration and the number of microorganisms and between deterioration and the moisture ratio. This makes it possible, as previously stated,[2] to predict the keeping quality of sugar by a preliminary bacteriological and chem ical analysis.

(2) From 3 to 6 bags of Cuban raw sugars, each of 10 different marks, with moisture ratios varying from 0.18 to 0.50, were stored under normal conditions in a large warehouse and were analyzed chemically and bacteriologically at the beginning and after four and eight weeks, respectively. There was a loss in polarization in most of the sugars at the end of each period, which was generally accompanied by a gain in reducing sugars and moisture content.

(3) There was a decided increase in the number of microorganisms per gram, especially during the first four weeks, which could be correlated, within certain limitations, with deterioration. In general, there were more microorganisms in the middle of the bag than at the surface. A large initial infection or rapid multiplication of microorganisms was responsible for an increase in deterioration.

(4) It has been shown that the use of superheated steam in the centrifugal will reduce the number of microorganisms more than 90 per cent and consequently may eliminate deterioration if the moisture ratio is likewise properly controlled.

[1] KOPELOFF, Nicholas. THE PREVENTION OF SUGAR DETERIORATION BY THE USE OF SUPERHEATED STEAM IN CENTRIFUGALS. *In* Jour. Indus. and Engin. Chem., v. 12, no. 9, p. 860–862, 1 fig. 1920.

[2] —— KOPELOFF, Lillian. OP. CIT., 1920.

FREEZING OF FRUIT BUDS

By FRANK L. WEST, *Physicist*, and N. E. EDLEFSEN,[1] *Assistant Physicist, Utah Agricultural Experiment Station*

INTRODUCTION

Killing frosts occur in the late spring and early fall over large areas of the United States, causing damage to the extent of several millions of dollars annually. The commonest method of protection is to heat the area by burning oil in pots distributed through the section that is endangered. Heating is resorted to on a large scale in the citrus fruit sections of California and less frequently elsewhere for the protection of such fruits as apples, peaches, and cherries. The success of this practice depends on the economical use of fuel and labor. If the predicted minimum temperature is lower than the "critical temperature" by an amount that exceeds the rise in temperature that the heaters will produce, or if the minimum temperature is above the "critical temperature," then the heaters should not be lighted. In order, therefore, to be able to tell when to light the heaters, it should be known how hardy the buds are. This paper gives the methods used and the results obtained from freezing more than 24,000 fruit buds, most of them being apples and peaches, and also the spring freezing temperatures and the yields of fruit in orchards near Logan, Utah, from 1913 to 1920.

THEORY OF INJURY DUE TO FREEZING

Pure water freezes at 32° F. Salts dissolved in water cause it to freeze at a lower temperature than this, the amount of the depression of the freezing point depending upon the nature of the salt dissolved and also upon the concentration of the solution. Thus, a 5 per cent common salt solution freezes at 27°, while a 30 per cent sugar solution freezes at only 29° F. W. H. Chandler[2] found that the expressed sap from fruit buds froze at 28° to 29° and in no case required a temperature below 28°. The sap from Elberta peach twigs, extracted in March, froze at 28.7°, while but two-thirds of the twigs of the same kind of fruit when subjected in March to a temperature as low as 10° froze. It is frequently found that some of the buds withstand temperatures as low as 20° and mature.

The more concentrated the aqueous solution, the lower is its freezing point, and in general the amount of the substance, especially if it is organic, that will dissolve in water is but slightly affected by the substances that are already in solution. This allows the possibility of a

[1] Messrs. J. Z. Richardson, W. E. Goodspeed, and Scott Ewing rendered valuable assistance with the laboratory and field work.

[2] CHANDLER, W. H. THE KILLING OF PLANT TISSUE BY LOW TEMPERATURE. Mo. Agr. Exp. Sta. Research Bul. 8, 309 p., 3 pl.: chart. 1913. Bibliography, p. 305–309.

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very concentrated solution, and each of these substances has its influence in lowering the freezing point of the water largely independent of the others. For these reasons, a rather low freezing point of a solution is possible. A very concentrated juice, therefore, in the buds would be expected to freeze at a fairly low temperature. In spite of this, however, the unusual hardiness of some buds to freezing is really surprising. The difference in sensitiveness to cold of different buds on the same branch and of the same buds at different stages of development may be in part due to the difference in quality and concentration of the cell sap.

TABLE I.—*Classified list of the "danger points" for various kinds of fruit as given by different authors*

Kind of fruit.	Petals closed but showing color.	In blossom.	Fruit setting.	Authority.
	°F.	°F.	°F.	
Apples	27	29	30	W. M. Wilson.[1]
	27	29	30	P. J. O'Gara.[2]
	27	29	30	W. H. Hammon.[3]
	25	28	28	Paddock and Whipple.[4]
	25	28	28	W. H. Chandler.[5]
Peaches	20	25	28	W. M. Wilson.[1]
	29	30	30	W. H. Hammon.[3]
	29	30	30	P. J. O'Gara.[2]
	22	28	28	Paddock and Whipple.[4]
	25	27	27	Garcia and Rigney.[6]
Cherries	22	28	29	W. M. Wilson.[1]
	29	30	30	P. J. O'Gara.[2]
	22	28	28	Paddock and Whipple.[4]
Pears	27	29	29	W. M. Wilson.[1]
	29	29	29	P. J. O'Gara.[2]
	28	29	29	W. H. Hammon.[3]
	25	28	28	Paddock and Whipple.[4]
Plums	30	31	31	W. M. Wilson.[1]
	30	30	31	P. J. O'Gara.[2]
	30	31	31	W. H. Hammon.[3]
	22	28	28	Paddock and Whipple.[4]
Apricots	30	31	32	P. J. O'Gara.[2]
	30	31	32	W. H. Hammon.[3]
	22	28	28	Paddock and Whipple.[4]
Prunes	30	31	31	P. J. O'Gara.[2]
	30	31	31	W. H. Hammon.[3]

W. H. Chandler [7] reports minimum temperature and the resulting damage by natural frost. He also reports his work on the artificial freezing of detached branches. Garcia and Rigney [8] placed self-registering minimum thermometers in the orchard. After a freeze the percentage of frozen buds was determined, and in the fall the yield of the orchard was obtained. Their work covered five years.

[1] WILSON, Wilford M. FROST. *In* Bailey, L. H., ed. Standard Cyclopedia of Horticulture. v. 3, p. 1283. New York, 1915.

[2] O'GARA, P. J. THE PROTECTION OF ORCHARDS IN THE PACIFIC NORTHWEST FROM SPRING FROSTS BY MEANS OF FIRES AND SMUDGES. U. S. Dept. Agr. Farmers' Bul. 401, p. 20. 1910.

[3] GARCIA, Fabian, and RIGNEY, J. W. HARDINESS OF FRUIT-BUDS AND FLOWERS TO FROST. N. Mex. Agr. Exp. Sta. Bul. 89, p. 5. 1914.

[4] PADDOCK, WENDELL, and WHIPPLE, Orville B. FRUIT-GROWING IN ARID REGIONS . . . XX, 395 p. illus. New York, 1910.

[5] CHANDLER, W. H. OP. CIT., p. 146.

[6] GARCIA, Fabian, and RIGNEY, J. W. OP. CIT., p. 51.

[7] CHANDLER, W. H. OP. CIT. 1913.

[8] GARCIA, Fabian, and RIGNEY, J. W. OP. CIT.

When liquids are cooled to their freezing points, if there be none of the solid material present, they rarely freeze. They may be cooled several degrees further and kept for days without solidification taking place. The introduction of as small an amount of solid as one-hundred-thousandth part of a milligram is sufficient to cause freezing to begin. The smaller the amount of liquid taken the easier it is to superfuse it, and liquids contained in capillary tubes will remain for long periods of time below their freezing point without solidification taking place. The fact that the juice of the buds is confined in small capillary spaces will help to explain in part the unusual hardiness of the buds and the great difference in hardiness of buds that appear to be very similar. This phenomenon explains why they may be cooled below their freezing points and be warmed again without ice separating.

A classified list of the "danger points," as given by various investigators, is presented in Table I.

METHODS AND APPARATUS

NATURAL FREEZES

Each spring, for the last seven years, standard minimum thermometers have been placed in especially prepared but simple shelters in fruit trees of various orchards near Logan, Utah, and were read the day after a minimum temperature of 32° F. or lower was experienced. A record was made of the yield of fruit of the orchard for the season. The results of this work are found in Table II.

ARTIFICIAL FREEZES

The first work consisted in freezing detached branches of fruit buds in the laboratory by means of a specially designed thermostat, the air surrounding the buds being cooled by means of common salt and ice and warmed with an incandescent electric light, which was maintained constant at an arbitrarily determined temperature in the usual way with a relay. The extent of the injury was determined by cutting the buds open and counting those that had been damaged and then calculating the percentage that had been frozen.

Branches of trees were bent down into a vessel surrounded by a second air chamber, the latter being surrounded by a mixture of ice and salt. The minimum temperature was noted, the branch was tagged, and the further development of the buds was observed and the yield of fruit determined.

This method was modified by having the buds cooled by means of evaporating liquid carbon dioxid instead of using ice and salt. A tank of liquid carbon dioxid was connected to a metal coil that surrounded the bud chamber. The very cold gaseous carbon dioxid cooled the bud chamber, thereby cooling the buds to the desired temperature.

The fourth method consisted in freezing the whole tree by surrounding and covering it with a two-walled metal vessel containing ice and salt. The apparatus is shown in Plate 80.

The factors that determine the amount of damage done and that need to be controlled in the experiment are:

1. The kind of buds.
2. Their stage of development.
3. The minimum temperature.
4. The humidity.
5. The duration of the freeze.
6. The rate of thaw.

The first three are of most importance. By keeping the other factors fairly constant and varying the fifth and sixth, little difference in the results was noted. In almost every case in nature, as well as in our experiments, the humidity just as freezing occurs is practically 100 per cent. Transpiration into a closed vessel will ultimately give this result, and the best desiccating agents will not keep the humidity down appreciably. This holds true also in the orchard simply by the cooling irrespective of the transpiration, because even in such a dry section as the arid West, with a humidity as low as 50 per cent and a cool spring day of perhaps 45° F. noon temperature, the dew point would be 27.5° F., which is about the temperature at which slight damage is caused. Where the humidity is higher, as it is in most places east of the Rocky Mountains and west of the Sierra Nevada Mountains, the dew would collect and the humidity would be 100 per cent even before the buds had cooled to the danger temperature. In all the work here reported the humidity was practically 100 per cent.

While the whole tree was being frozen, several minimum thermometers were suspended at different places in its branches, and the air was stirred by an electric fan driven with storage batteries. The humidity was determined by a continuous reading hygrometer, and the rate of thaw and duration of freeze were recorded by means of a thermograph that was placed in the branches.

The cost of the different methods is about the same for freezing the same number of buds. Adjoining limbs and adjacent trees were thinned to the extent that the branch or tree had been thinned by the frost, and the yields in the fall were noted for comparison. A greater variation in the factors, and thus a greater number of different experiments, can be secured for the same expenditure by freezing the branches on the tree rather than the whole tree.

The results of the natural and artificial freezing experiments are presented in Tables II to IV.

TABLE II.—*Temperatures produced and percentage of buds killed by artificial freezing*

Kind of fruit.	Number of buds.	Development.	Temperature.	Percentage of damage.
			°F.	
Ben Davis apples	110	Showing color	22.5	88
	1,935	do	25	45
	2,172	Full bloom	24	81
	101	do	24.5	56
	813	do	25	54
	1,828	do	26	16
	1,490	do	26	100
	28	do	26.5	36
	127	do	27.5	54
	4,217	do	28	0
	12	do	28.5	0
	29	Fruit setting	25.5	93
	49	do	26.5	40
	40	do	26.5	23
	33	do	27.5	21
	48	do	27.5	59
	55	do	27.5	62
	58	do	28	46
Elberta peaches	1,715	Showing color	17.5	64
	1,846	do	18	75
	35	do	20	66
	675	do	22.5	76
	111	do	22.5	76
	277	do	24	89
	361	do	24	79
	514	do	25	96
	380	do	25	74
	586	do	25	77
	1,195	do	25	97
	189	do	26	80
	372	do	27.5	79
	349	Full bloom	22	100
	38	do	24	63
	22	do	24	64
	42	do	25	58
	62	do	25	28
	35	do	25	72
	1,061	do	25	65
	42	do	26	40
	.....	do	26	48
	355	do	26	78
	749	do	26	54
	194	do	26	57
	37	do	27	0
	27	do	27	0
	507	do	27	55
	.....	do	28	55
	.....	do	28	33
	80	Fruit setting	24.5	30
	.....	do	25	100
	16	do	26	75
	70	do	26.5	48
	49	do	27	75
	78	do	27.5	56
	.....	do	27.5	48
	.....	do	28	43
	.....	do	29	33.3

TABLE III.—*Temperatures produced and number of mature fruits harvested by artificial freezing*

Kind of fruit.	Number of buds.	Development.	Temperature.	Number of fruits harvested.
			°F.	
Ben Davis apples	30	Full bloom	20	4
	38	do	20	9
	18	do	20	2
	60	do	20	0
	19	do	21	0
	39	do	22	3
	36	do	22	4
	12	do	23	0
	19	do	23	0
	37	do	23	0
	119	do	23	14
	32	do	24	4
	149	do	25	8
	64	do	25	3
	55	do	25	0
	44	do	25	0
	45	do	25	0
	89	do	28	7
	81	do	28	9
	35	do	28	7
	45	do	28	7
	71	do	28	0
Control limbs, untreated	108	Full bloom	.....	13
	74	do	.....	8
	122	do	.....	15
	63	do	.....	10
	141	do	.....	17
Ben Davis apples	71	Fruit setting	20	0
	52	do	25	0
	87	do	25	5
	64	do	25	0
	88	do	25	0
	105	d	25	0
	106	do	28	8
	69	do	28	0
	68	do	28	3
	69	do	28	0
	133	do	28	0

TABLE IV.—*Result of natural freezes*

Kind of fruit.	Showing color.	Full bloom.	Fruit setting.	Percentage killed.
Apples	28, 25, 27	24, 28	28, 28. 5	32
	27. 5, 27. 5	30. 5	31. 5, 30	8
	26	.....	.....	5
	.....	26	.....	41
	.....	.....	30	0
	.....	.....	29	0
	27	.....	.....	0
	28	.....	.....	0
	29	.....	.....	0
	.....	.....	28	0
	26, 25	32	.....	0
	29, 29, 30	.....	32	0

TABLE IV. *Result of natural freezes*—Continued

Kind of fruit.	Showing color.	Full bloom.	Fruit setting.	Percentage killed.
Prunes	22	.....	.....	0
	.....	26	.....	0
	.....	26	.....	0
Sweet cherries	22	.....	.....	44
	.....	.....	26	0
	.....	.....	26	0
	26, 25	.....	32, 31	0
	29, 25, 27	.....	.....	0
	30	.....	.....	0
	.....	26	.....	53
	29, 29	.....	.....	22
	30	.....	.....	30
	31, 32	.....	.....	0
	.....	25, 30	.....	50
Sour cherries	22	.....	.....	61
	22	.....	.....	23
	.....	26	.....	0
	.....	26	.....	10
	.....	26	.....	48
	26	.....	.....	20
	31, 23, 32, 25, 30	.....	.....	0
Elberta peaches	26	.....	.....	0
	.....	32	.....	0
	25	.....	.....	0
	.....	32	.....	0
	28	.....	.....	0
	25	.....	.....	0
	.....	30	.....	0
	.....	.....	30	0
	24	.....	.....	54
	22	.....	.....	36
	.....	26	.....	0
	27. 5, 27. 5	30. 5	31. 5, 30	56
Apricots	29, 25	.....	.....	0
	27, 30	.....	.....	20
	.....	26	32	20
	.....	22	.....	55

SUMMARY

(1) Efficient orchard heating demands an economical use of labor and fuel and requires knowledge of the temperatures that cause injury to the buds.

(2) This paper contains the results of seven years' experiments in freezing 24,000 apple, peach, cherry, and apricot buds, together with a record of the natural freezes that occurred in the orchards near Logan, Utah, during the same period.

(3) Ben Davis apple buds in full bloom have experienced temperatures of 25°, 26°, and 27° F. without injury, but 28° usually kills about one-fifth. Twenty-nine degrees or above are safe temperatures. Twenty-five degrees kills about one-half and 22° about nine-tenths. On several

occasions, however, apples matured on branches that experienced 20° when the buds were in full bloom.

(4) With Elberta peach buds in full bloom, 29° F. or above are the safe temperatures, because even though occasionally 26°, 27°, and 28° do no damage, yet on most occasions 28° will kill from one-fourth to one-half. Twenty-six degrees kills about one-half of them and 22° about nine-tenths. Temperatures as low as 18° have failed to kill all of them.

(5) With sweet cherry buds in full bloom, 30° F. is the safe temperature; 25°, 26°, 27°, 28° have done no damage; but 29° usually kills about one-fifth. Twenty-five degrees usually kills about one-half, and when the buds were showing color 22° killed only two-fifths of the buds.

(6) Sour cherries are hardier than the sweet varieties. When the buds were showing color 23° F. did not harm them, and when they were in full bloom 26° killed but one-fifth and 22° only two-fifths of them.

(7) With apricots, 29° F. is the safe temperature; 26° and 27° killed about one-fifth and 22° killed one-half. They are fairly hardy, but they bloom so early that they are frozen oftener than any of the other fruits studied in the experiments.

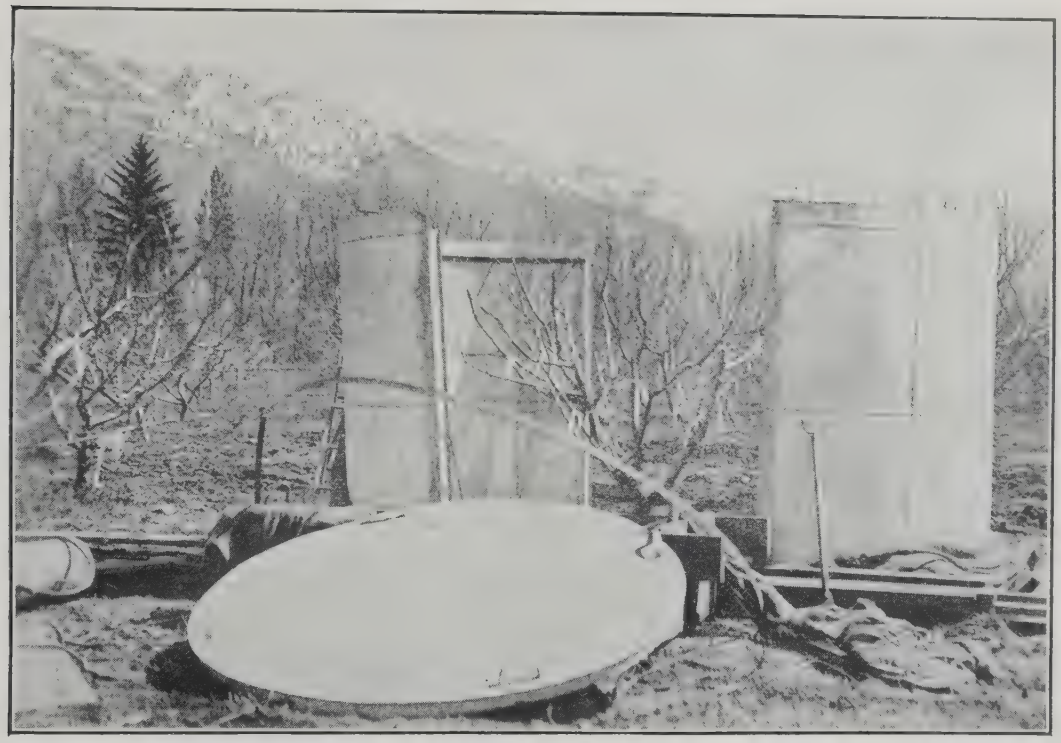
(8) The foregoing figures refer to the buds when in full bloom. Starting from this stage, the earlier the stage of development the hardier the buds are; and in general, when the fruit is setting the injury is from 5 to 10 per cent more than when they are in full bloom.

(9) Sour cherries are the hardiest, and then follow in order apples, peaches, apricots, and sweet cherries.

(10) The fact that the same branch of buds will on one occasion experience 27° F. with 25 per cent injury and on another occasion take the same temperature with no injury is no doubt due to the fact that the juice is contained in capillary cells and supercooling results—that is, the buds are cooled below the freezing point of the juice without the freezing taking place. The great difficulty of killing all the buds even at extremely low temperatures is due to the same cause together with the fact that the cell sap may be very concentrated. Differences in the hardiness of the different kinds of buds and also of the same buds at different stages of development is due to differences in quality and concentration of the cell sap.

PLATE 75

Apparatus for freezing entire tree.



EFFECT OF VARIOUS CROPS UPON THE WATER EXTRACT OF A TYPICAL SILTY CLAY LOAM SOIL

By G. R. STEWART, *Chemist, Hawaiian Sugar Planters' Association*, and J. C. MARTIN, *Assistant Chemist, California Agricultural Experiment Station*

The senior author has previously reported a series of investigations carried on at the California Agricultural Experiment Station upon the changes which took place in the water extracts from a group of selected soils. These consisted of six silty clay loams and seven fine sandy loams. All were typical soils brought from various places in California and represent a considerable range of past treatments and some variations in known productive capacity. A large quantity of each soil was brought to the Experiment Station at Berkeley, where it was sifted, mixed, placed in two uniform containers, and afterwards kept under controlled conditions. A crop of barley was raised upon all the soils during the first year of the experiment in order to bring them into a somewhat comparable state of tilth. During the second season one container of each soil was cropped and the other was maintained as an uncropped duplicate. Notable differences were found in the amounts of water-extractable constituents from the cropped and the uncropped soils. The water-soluble nitrates, calcium, potassium, and magnesium were generally higher in the uncropped soils. Considerable differences were also observed in the amounts of water-soluble constituents extracted from the different uncropped soils. Further details of the experimental methods and of the results obtained may be found in the original publication.[1]

The conclusion from our previous work, that barley reduces the nitrates of soils to a low and fairly uniform magnitude independently of the soils' crop-producing power and also tends to reduce the amounts of other water-extractable constituents, seemed to require that the observations be extended to include the effects of other crops. It was also deemed desirable to study the effect of varying numbers of plants in accelerating the changes observed and if possible to ascertain the rate of movement of water-extractable substances through the soil.

The experimental work consists of two separate studies, one to cover the specific effect of different types and numbers of plants, the other to shed light on the movement of solutes through the soil.

[1] STEWART, Guy R. EFFECT OF SEASON AND CROP GROWTH IN MODIFYING THE SOIL EXTRACT. *In* Jour. Agr. Research, v. 12, no. 6, p. 311–368, 24 fig., pl. 14. 1918. Literature cited, p. 364–368.

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EFFECT OF TYPE AND NUMBER OF PLANTS

A large portion of Yolo silty clay loam soil was sifted into a group of eight containers. Each container was of the same size as those previously used, 30 inches wide, 60 inches long, 18 inches deep, and held approximately 1,800 pounds of soil.

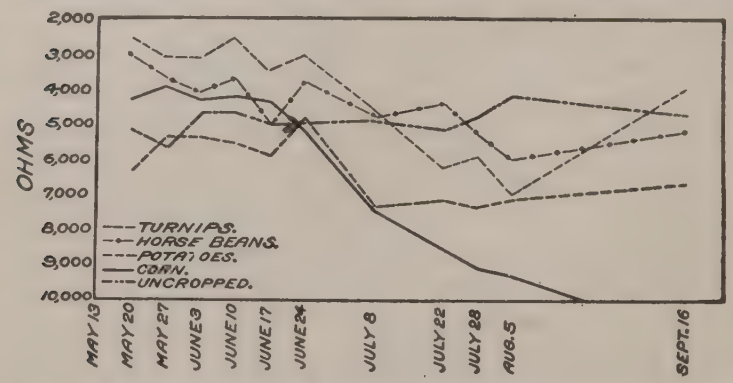


FIG. 1.—Decrease of water-soluble nutrients from the growth of various crops, as shown by increases in specific resistance. Crops were planted May 13, and soil was sampled on dates given.

One container was planted to Golden Ball turnips, one to horse beans, one to Early Golden Bantam corn, one to Burbank potatoes, and three to barley, the latter having, respectively, 24, 50, and 72 plants each. In addition, one container was left uncropped as a control.

Water extractions were made at intervals of one to two weeks throughout the major portion of the growing season. This period extended from the middle of May to the end of September. All the crops except the corn matured normally. The cool nights of the San Francisco Bay region prevent corn planted in the spring from maturing till late in the fall. The results with this crop, however, were of such a nature that observations thereon became unnecessary after the maturation of the other crops and were accordingly discontinued at that time.

The extractions were made in the proportion of 1 part of soil to 2 parts of water. The mixture was triturated in a mortar for three minutes and then filtered upon a medium grade of semi-quantitative paper in an ordinary funnel. The first portions were poured back until reasonably clear filtrates were obtained. The conductivity of this solution was then determined by the Wheatstone bridge and is expressed in the graphs as ohms of specific resistance. An increase of resistance, therefore, represents a lowering of the concentration of electrolytes present. Work performed in this laboratory on similar solutions has shown that this method gives results which are comparable to those obtained by accurate

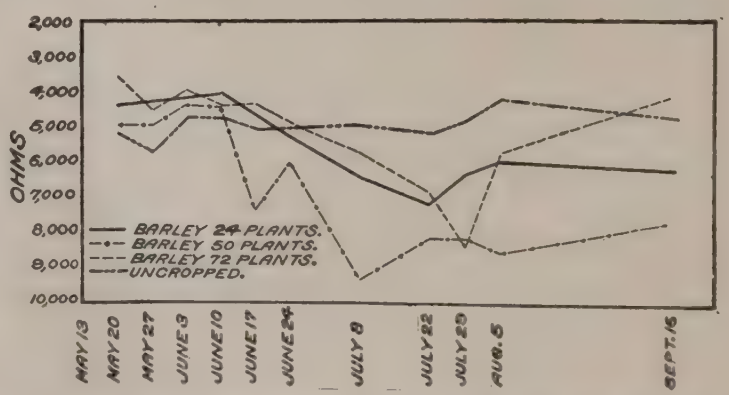


FIG. 2.—Decrease of water-soluble nutrients from varying numbers of barley plants, as shown by increase in specific resistance. Crops were planted May 13, and soil was sampled on dates given.

determinations of total solids. The results of these conductivity determinations are plotted in figures 1 and 2.

Here we find that all the crops have reduced the concentration of the water extracts during the middle of the growing season. It is interesting to note in the cases of the barley crops that even the smallest number of plants was sufficient to effect a substantial reduction of water-extractable solutes by the time the plants had become well established. The uncropped soil, on the other hand, maintained a remarkably uniform concentration throughout the period of observation.

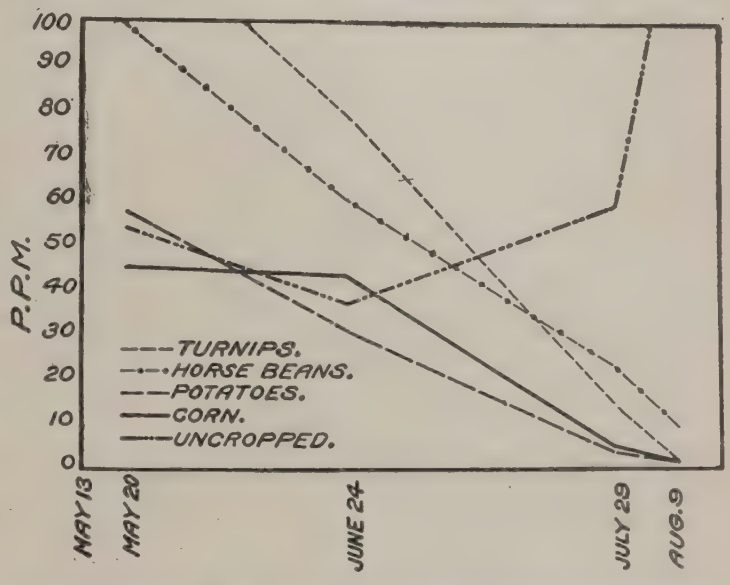


FIG. 3.—Decrease of water-soluble nitrates from the growth of various crops. (Graphs=½ NO_3.) Crops were planted May 13, and soil was sampled on dates given.

Nitrates were determined at a few periods, and these results are given in graphs 3 and 4.

Here we see that each crop at maturity had depressed the soil's nitrate content to a minimum. The uncropped soil constantly remained on a higher level.

MOVEMENT OF SOLUTES THROUGH THE SOIL

In this experiment two containers of the same soil were placed in the greenhouse and buried in the ground, level with the floor for heat insulation. Two rows of sugar beets were planted across one end of one container. These were spaced 6 inches apart in the row and 9 inches between rows. The remainder of the container, some 40 inches in length, was left bare. Two rows of barley were planted in one end of the other container. The plants were spaced 6 inches apart and 6 inches between rows. This left 40 inches of unoccupied ground.

The crops were started in December and were allowed to grow until the following March. By that time the beets were about 2 inches in diameter and the barley was fully headed.

Periodic observations of the concentration of the soil solution were made by means of freezing-point determinations. Two samples were always taken from each container, one from between the rows of beets or barley and the other near the bare end of the tank. The freezing-point depressions for both groups of samples are given in figure 5. The last sample in April represents the condition we have previously observed in soils when barley had made about the same growth.

At this time a longitudinal section was cut in the soil, and the root extension of both crops was studied. With the sugar beets it was found that a thick, matted growth of fine rootlets extended from the second row of beets to the extreme end of the container, 41 inches in all. Many of these rootlets were branches from the main fleshy feeders. These extended laterally throughout the bare end of the tank. The main barley roots were found to extend 32 inches from their plant sources with the finer rootlets extending 1 foot further toward the bare end of the container. A portion of the roots also extended to the bottom of the container and ran almost to the end wall.

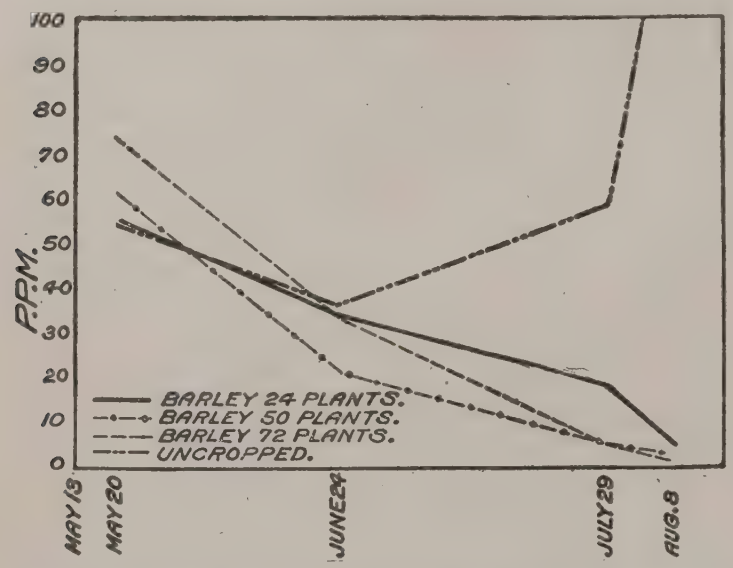


FIG. 4.—Decrease of water-soluble nitrates from varying numbers of barley plants. (Graphs=½ NO_3.) Crops were planted May 13, and soil was sampled on dates given.

The soil solution during the early stages of growth of both barley and beets appeared to have a significantly lower concentration in the near neighborhood of the plants than at a distance therefrom. It was not until the early part of April when the plants had reached a considerable size that the soil solutions in the cropped and uncropped ends of the containers approached each other in concentration. Unfortunately for the original objective of the experiment, the roots of the plants, in both cases, appear to have penetrated the soil mass of the bare ends of the containers about as rapidly as the concentration of the soil fell off.

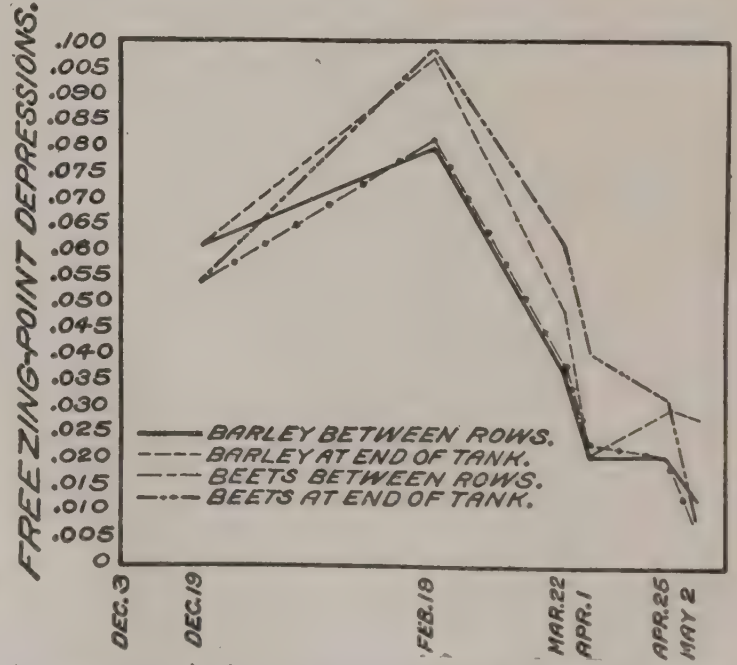


FIG. 5.—Decrease in the concentration of soil solution shown by freezing-point depression. Crops were planted December 3, and soil was sampled on dates given.

There is, therefore, no proof here, either as to the rate of translocation or the distance through which the soil solutes may move by diffusion. But since the losses of concentration of the soil solution appear to be somewhat proportional to root penetration, it would seem probable that the rate of movement of solutes through the soil is less than the rate of growth of the roots of normal barley and beets.

CONCLUSIONS

The gain in specific resistance and the decline in nitrate content of the water extracts of soils planted to different crops warrant us in extending the conclusions heretofore drawn from observations of the effects of barley. It is clear that the phenomena noted are not peculiar to the barley plant but are characteristic of all the plants tested and probably apply to all chlorophyll-bearing plants which root in the soil. The extent of the reduction of concentration observed is variable with different crops. We may not put too much stress upon the magnitudes of these differences, however, because of the obvious differences in growth habits and life history of the plants considered. It is interesting to note, however, that corn which is commonly regarded as a "gross feeder" in ordinary fertilizer practice has increased the specific resistance of the water extracts more rapidly and completely than the other plants.

The second experiment sheds little light on the rate of movement of solutes toward the plant roots. Inasmuch, however, as reductions in concentration of water extracts of soil at a distance from growing plants did not take place until that portion of the soil had become filled with roots, it would seem that rapid and extensive movements of soil solutes are probably not an incident of normal plant absorption.

SUMMARY

(1) The effect of crops of corn, horse beans, potatoes, turnips, and barley upon the water extract from a typical silty clay loam was studied throughout the growing season.

(2) All the crops discussed in this paper reduced the concentration of the water extract during the height of the growing season.

(3) The nitrate content of the soil was reduced to a very low figure by all crops.

(4) An experiment in which the concentration of the soil solution was studied by means of observations of freezing-point depressions in the immediate vicinity and at a distance from the plants showed that concentrations are not significantly reduced until the portion of the soil sampled is filled with plant roots.

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ANOTHER CONIDIAL SCLEROSPORA OF PHILIPPINE MAIZE

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Each year in the Philippine Islands the valuable maize crop suffers very severe losses from the destructive activities of downy mildew (*Sclerospora* spp.). While the writer was studying this disease during the past two years his attention was naturally directed to the question whether the widespread destruction of maize throughout the thousand-mile extent of these scattered islands was due in all cases to the same species of fungus. A comparative study of material collected from many parts of the provinces of Batangas, Laguna, and Rizal in the island of Luzon, where the disease is most serious and where it was studied most intimately, showed that in all cases the same causal fungus was involved. This species of downy mildew was described in an earlier paper (*12*)[1] as *Sclerospora philippinensis*. It was only natural to suspect that some of the abundant Philippine wild grasses related more or less closely to maize would be found to harbor this or other Sclerosporas. As on the widely distributed wild grass *Saccharum spontaneum* L. (Pl. 77, A) the oogonial stage of a Sclerospora had been very commonly encountered in great abundance, this grass was obviously an object of suspicion. In Luzon, however, despite extensive search, no conidial stage was seen on this host.

During a trip to the more southern Visayan Islands of Cebu, Bohol, and Leyte, in which maize is a crop of very great importance, the writer found that there, also, the maize plantings were suffering heavy losses from downy mildew. As no microscope was carried, no study of the causal organism was made at night during the period of conidium production. However, inasmuch as the symptoms and the general effect of the downy mildew were the same in these southern islands, the writer inferred that the causal organism was that which he had found so widely distributed on maize throughout the northern island of Luzon. Also the wild grasses of these southern islands were carefully examined as possible

[1] Reference is made by number (italic) to "Literature cited," p. 684.

hosts for downy mildew. After long search a clump of buganğ grass (*Saccharum spontaneum*) heavily infected by a conidial Sclerospora was discovered by Mrs. Weston. Continued hunting brought the fungus to light on the same host in two other places, all three cases being encountered in the rugged interior uplands of Cebu (Pl. 76), which lie between Carcar and Barili. In the island of Leyte, also, this Sclerospora was again found on buganğ grass on a hillside about three miles from Baybay. No other cases of downy mildew either on this or on other hosts were seen. Later, in a field of native sugar cane near Guadelupe cemetery outside the town of Cebu, a single clump of cane was found infected with the conidial stage of a Sclerospora.

The infected plants of *Saccharum spontaneum* and sugar cane were transplanted to Los Baños, Luzon, for further study (Pl. 77, B). There a comparison of living material taken from these plants during the optimum time of nocturnal conidiophore production showed that this downy mildew from the southern islands was different from that previously studied in Luzon. This discovery necessitated a revision of all available material in order to determine whether or not other forms had been previously overlooked under the assumption that the collections were all of the same form so commonly found in Luzon. Accordingly, living material from maize, teosinte, and sorghum from the college plots and from native fields in Batangas and Laguna provinces was compared with the living material from the plants of *Saccharum spontaneum* and sugar cane brought from Cebu. Dried, preserved, and mounted specimens from maize collected in various parts of Luzon were compared with similar specimens from maize obtained in various localities in Cebu, Bohol, and Leyte. This survey showed clearly that all the material so far encountered fell into one or the other of two distinct species—one, the form with shorter, broader conidia found on maize, etc., in Luzon and previously described as *Sclerospora philippinensis*, and the other, which will be called *Sclerospora spontanea*, characterized by longer, narrower conidia, and found on maize, buganğ grass, and sugar cane in the Visayas. Once this point had been established, a comprehensive study was made of the two species to determine the resemblances and differences between them in morphological and physiological characteristics.

COMPARATIVE STUDY OF SCLEROSPORA PHILIPPINENSIS AND SCLEROSPORA SPONTANEA

FIELD CHARACTERISTICS

On maize, as observed in the field in the more southern islands and in Luzon, the two species are apparently identical in their destructiveness to the crop as a whole and also in their effect on the individual plants. It is possible that quantitative studies of essentially similar fields infected by the separate species would show some slight differences, but in general appearance there is no distinction whatever between the two.

PHYSIOLOGICAL CHARACTERISTICS

Several varieties of maize grown in sterile soil and under controlled conditions preventing contamination were infected with spores produced on the living plants of buganğ grass (*Saccharum spontaneum*) and sugar cane brought from Cebu. Parallel inoculations were made also with *Sclerospora philippinensis*. No difference was apparent either in symptoms or in the virulence of the resulting infection. Similar experiments with seedlings of cultivated wheat, Setaria, Pennisetum, and several species of wild grasses, including the very common aguinğay (*Rottboellia exaltata* L.), anias (*Andropogon sorghum* var. *halepense* L.), cogon (*Imperata cylindracea* L.), and tigbee (*Coix lachryma-jobi* L.), using the long, narrow conidia of the southern species, were as uniformly unsuccessful as they had been with *Sclerospora philippinensis* (*12*). Seedlings of teosinte (*Euchlaena luxurians* Schrad.) and the wild grasses, *Saccharum spontaneum* and *Miscanthus japonicus* (Thunb.) Anders., were successfully inoculated with both forms. No seeds of sugar cane were available for planting. Had there been, there is little doubt in the mind of the writer that infections in this case also could have been obtained. A more detailed account of these inoculation experiments will be given in a later paper. It should be said here, however, that the effect of the Sclerosporas varied with the different hosts, being most destructive on maize and least so on buganğ grass; but the characteristic production of conidiophores took place with uniform regularity at night on all (Pl. 78, B).

A comparative study of material of *Sclerospora spontanea* on these different hosts showed that the distinguishing morphological characteristics of the fungus had not been altered in any way. Moreover, even after transition from one host to another through several generations, the species remained constant and in no way approached *S. philippinensis*. In like manner, after inoculating various hosts and passing through several generations, *S. philippinensis* also was quite unchanged and showed no tendency to approach the long-spored form.

The writer considers it quite possible that an exact statistical study of large numbers of individuals infected by each of the two fungi would reveal some slight quantitative difference in the area bearing conidia, or in the rate of growth of hyphae through the host, or in some other aspect not at once apparent to an ordinary comparative examination. It should be noted here, however, that there is certainly no noticeable physiologic difference between the two in virulence, range of hosts, or general course of the resulting disease they produce.

MORPHOLOGICAL CHARACTERISTICS

Therefore, because the two forms differ morphologically rather than physiologically, they were carefully compared in order to determine whether the points of difference were sufficiently stable and well marked

to establish the long-spored form as a species distinct from *Sclerospora philippinensis*.

MYCELIUM.—In morphological characteristics, extent, and relation to the host tissue, the mycelium of the two fungi showed no distinctions sufficiently marked or unvarying to warrant their use as a basis of separation. However, the club-shaped hyphae (conidiophore initials) which grow out through the stomata and develop into conidiophores are different in the two forms, those of the long-spored Sclerospora being markedly longer, more slender, and more irregular.

CONIDIOPHORES.—In general appearance the conidiophores of the two Sclerosporas are noticeably dissimilar, those of the Visayan form being markedly longer, more slender, and more spreadingly branched than those of *Sclerospora philippinensis*. On analyzing this dissimilarity the details of difference discussed in the following paragraphs are apparent.

The basal cell of the Visayan Sclerospora is very long (Pl. 79, A, D, E, F, H), strikingly longer than that of *Sclerospora philippinensis*. The length (140 to 260 μ) is greater not only actually but also relatively, for even in the unusual cases when it is less conspicuously long (Pl. 79, G) the basal cell of the Visayan Sclerospora always exceeds or at least equals in length that part of the main axis extending from the terminal septum of the basal cell to the origin of the primary branches. In *S. philippinensis*, the basal cell is always shorter than this part of the main axis. Moreover, the basal cell of the Visayan Sclerospora is much more slender, usually 5 to 8 μ at its narrowest diameter, and much less knobbed or swollen at its base (Pl 79, A, D, E, F, H) than is the basal cell of *S. philippinensis*.

The main axis of the Visayan Sclerospora expands more abruptly above the basal cell and then constricts more distinctly (Pl. 79, A, D) just below the branches than in *Sclerospora philippinensis*. The greatest diameter (22 to 32 μ), which usually slightly exceeds that of *S. philippinensis*, is thus placed, not just below the branches (as in *S. philippinensis*), but some distance lower (Pl. 79, A, D, G, H).

The branches of the Visayan form generally are less constricted at their point of origin, are of more uniform diameter, and are straighter, less ascending, more spreading, and do not recurve, but stand out from the main axis more stiffly. They are characteristically longer and more slender, but, even if short and crowded, they stand out more stiffly than in *Sclerospora philippinensis*. Although varying considerably in both species, the number of conidia produced on conidiophores is approximately the same in *S. spontanea* and in *S. philippinensis*. In the former, 32 to 48 are commonly borne, although as many as 88 or as few as 12 may less frequently occur.

The sterigmata also are straighter, less recurved, and stand out more stiffly than in *Sclerospora philippinensis*, and, usually they are longer (about 13 μ). It should be noted, however, that the length varies with

the extent of the branch system, since in cases where this is reduced and the primary branches or even the main axis give rise directly to sterigmata, these sterigmata are much larger (Pl 79, B) than they are when arising from quaternary or tertiary branches as the ultimate structures of an elaborate system (Pl. 79, A).

As a result of such differences, the conidiophore top of the Visayan Sclerospora has a more spreading, expanded appearance; and the long axes of the branches, the sterigmata, and the conidia borne on them stand out from the main axis like rays of a partly opened fan. In *Sclerospora philippinensis*, on the contrary, the conidiophore top is more compact and less spreading, the axes of branches, sterigmata, and conidia being all approximately parallel to each other and to the main axis.

These differences in the conidiophores of the two fungi are, on the whole, relative rather than absolute and are influenced to some extent by such environmental conditions as the depth and persistence of the layer of dew in which they develop. Even these distinctions, however, could be used as more absolute and less relative criteria if a very large number of measurements of all parts of the conidiophores were made and assembled to give an adequate quantitative impression. Even from the qualitative rather than quantitative point of view, moreover, these differences, although relative, are constant and distinct, and it should be emphasized that they persist when the two fungi, developing under exactly parallel circumstances on sister plants of the same age, grown side by side under as nearly the same conditions of temperature, soil, dew deposition, etc., as it was possible to obtain, were compared by nightly examinations for several weeks.

Conidia.—Among the Peronosporaceae as a whole the characteristics of the conidia have been found to be the most valuable basis for distinguishing species. This applies equally well to these two Sclerosporas, since their conidia not only differ markedly and constantly in shape and size but also remain relatively unaffected by changes in environment and hosts.

In shape, the conidia of the Visayan Sclerospora are at once distinguished from those of *Sclerospora philippinensis*. They are not only much more elongate but much more slender as well, the length being frequently two or even three times the diameter. Consequently they range from very elongate ovoid and obovoidal bodies to long narrow, round-ended cylinders, but they are most commonly very elongately ellipsoid in shape. A clearer idea of these variations may be gained from Plate 79, I, J, K.

In such features as the rounded apex devoid of any papilla, the blunt base with its apiculus of attachment, the hyaline, granular content, and the thin wall, the conidia correspond to those of *Sclerospora*

philippinensis. As in the case of the latter species also, germination is invariably by the protrusion of one or more germ tubes (Pl. 79, I, J, K).

In size, the conidia of the Visayan Sclerospora are very variable. With respect to such widely varying bodies as the spores of this and other genera of Peronosporaceae, recent investigations have shown that it is no longer possible to delimit a species adequately by the extremes or averages of a few measurements. Rather, there is required the assembling and presentation in tables and graphs of a sufficiently large number of representative measurements to give a quantitative as well as a qualitative expression of the conidial characteristics of the species.

Accordingly, in order to obtain data adequate to identify the Visayan form and to furnish a basis for comparing it with others, 700 conidial measurements were made. These comprised measurement groups of 100 conidia from each of the two sugar-cane and the four *Saccharum spontaneum* plants from Cebu, and from one maize plant inoculated from the latter.

The conidia were taken from the leaves of the host at night during the optimum period of conidia production—from 2 to 4 a. m.—mounted in dew, and measured immediately.

Since, on examination, the seven measurement groups were found to agree in all essential particulars, they were combined into the total of 700. For the purposes of comparison, 700 measurements of *Sclerospora philippinensis* were secured in like manner.[1] Of these, 300 were new ones made of fresh conidia from teosinte and sorghum found infected in the college plots and from *Saccharum spontaneum* seedlings artificially inoculated from maize. All these groups were compared, found to agree, and grouped into the total of 700.

In making these measurements, care was taken to include every conidium in a marked area of the microscope field as the slide was moved along by the mechanical stage. Only those conidia obviously injured or those still attached to the conidiophores were excluded. The divisions of the eyepiece equaled approximately 1.8 μ, and, with the magnification used, it was possible to estimate with fair accuracy to one-third of a division, or to about 0.6 μ. Consequently, the measurements are exact to this extent—that is, the conidium recorded as 32 μ in length may as well be 31.4 μ or 32.6 μ instead of exactly 32 μ but not, in all probability, 31 or 33 μ. With a large number of spores such differences tend to equalize themselves. As a result, the measurements presented here may be considered as adequately representing the characteristics of the conidia of the species involved.

[1] The writer wishes to take this opportunity to call attention to an error in the tabulation of the previous spore measures of *Sclerospora philippinensis* (12, p. 110). In the table of length, the conidia measuring 41 to 42.9 μ should be 23 in number instead of 24.

The measurements are summed up in Table I and are presented in graphic form in figure 1. In addition, the biometric characteristics of the two species are given in Table II. In making the calculations, the directions and formulae of E. Davenport (*3*) and C. B. Davenport (*2*)

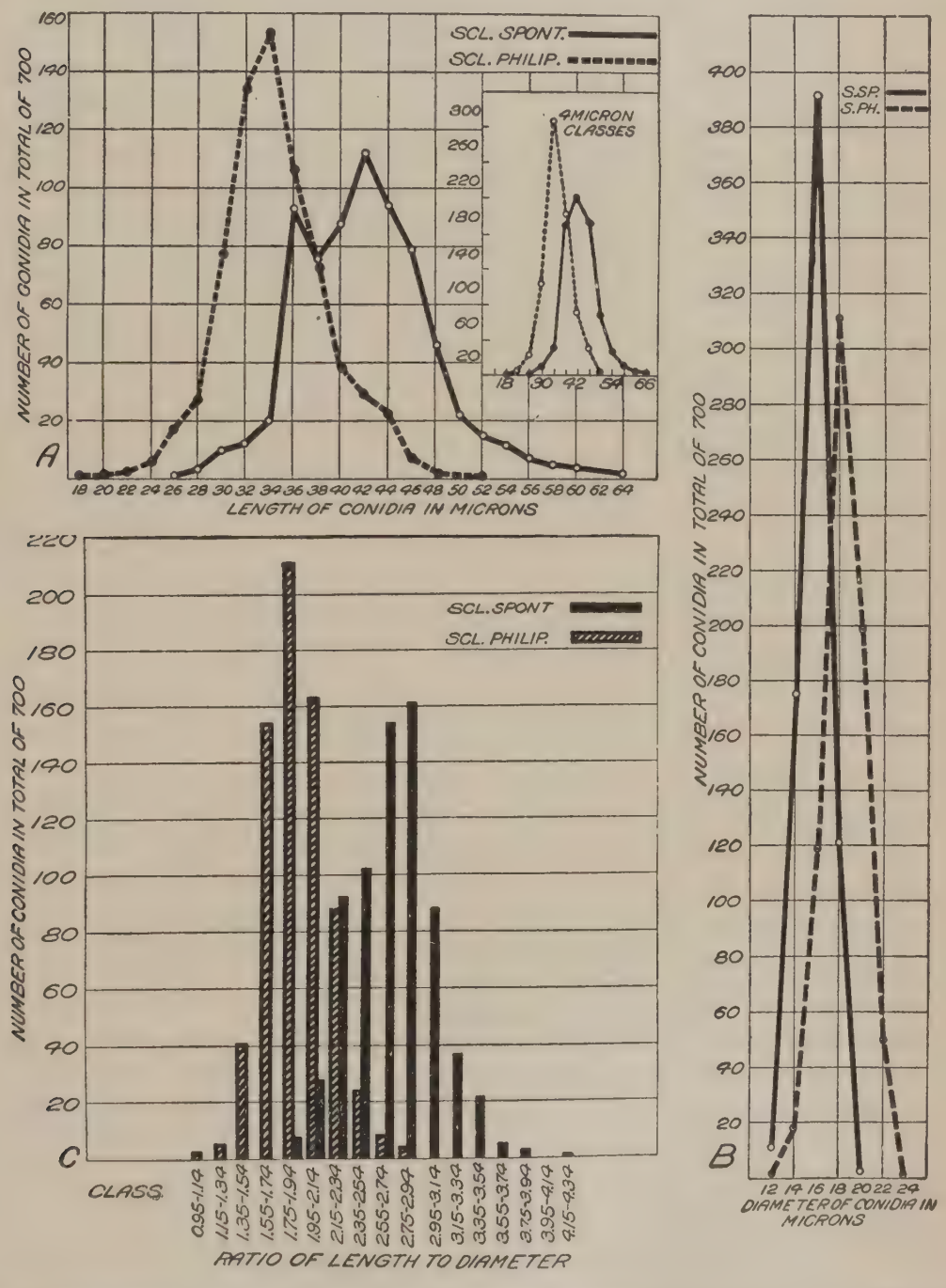


FIG. 1.—Comparison of the sizes of 700 conidia of *Sclerospora spontanea* with 700 conidia of *S. philippinensis*; *A*, variation of conidia in length; *B*, variation of conidia in diameter; *C*, ratios of length to width of conidia arranged in classes.

have been followed. The writer makes no pretense to a comprehensive biometric study of the two Sclerosporas but has used this method solely as a means to the end of presenting the accompanying data as a basis of comparison between these and other species.

TABLE I.—*Summarized measurements of conidia of Sclerospora spontanea and Sclerospora philippinensis*

Length.			Diameter.			Length over diameter.		
Classes.	Number of conidia in 700.		Classes.	Number of conidia in 700.		Ratio classes.	Number of conidia in 700.	
	S. spontanea.	*S. philippinensis.*		*S. spontanea.*	*S. philippinensis.*		*S. spontanea.*	*S. philippinensis.*
μ.			μ.					
17 to 18.9...	.....	1	11 to 12.9...	11	2	0.95 to 1.14...	.....	2
19 to 20.9...	.....	1	13 to 14.9...	175	18	1.15 to 1.34...	.....	5
21 to 22.9...	.....	2	15 to 16.9...	391	119	1.35 to 1.54...	.....	41
23 to 24.9...	.....	5	17 to 18.9...	121	311	1.55 to 1.74...	.....	154
25 to 26.9...	1	17	19 to 20.9...	2	199	1.75 to 1.94...	7	211
27 to 28.9...	3	27	21 to 22.9...	.....	50	1.95 to 2.14...	28	163
29 to 30.9...	10	77	23 to 24.9...	.....	1	2.15 to 2.34...	92	88
31 to 32.9...	12	134				2.35 to 2.54...	102	24
33 to 34.9...	20	153				2.55 to 2.74...	154	8
35 to 36.9...	93	107				2.75 to 2.94...	161	4
37 to 38.9...	76	75				2.95 to 3.14...	88	
39 to 40.9...	87	39				3.15 to 3.34...	37	
41 to 42.9...	112	29				3.35 to 3.54...	22	
43 to 44.9...	94	23				3.55 to 3.74...	5	
45 to 46.9...	79	7				3.75 to 3.94...	3	
47 to 48.9...	46	2				3.95 to 4.14...	0	
49 to 50.9...	22	0				4.15 to 4.34...	1	
51 to 52.9...	15	1						
53 to 54.9...	12							
55 to 56.9...	7							
57 to 58.9...	5							
59 to 60.9...	4							
61 to 62.9...	0							
63 to 64.9...	2							

TABLE II.—*Biometric constants of the conidia of Sclerospora spontanea and Sclerospora philippinensis*

LENGTH

Species.	Mean.	Median.	Mode (approximate).	Standard deviation.	Coefficient of variability.
	μ.	μ.	μ.		
S. spontanea.....	42.07±0.145	41.86±0.142	41.43	5.672±0.102	13.48±0.247
S. philippinensis..	34.52± .113	34.12± .142	33.32	4.439± .080	12.86± .235

DIAMETER

	μ.	μ.	μ.		
S. spontanea.....	15.79±0.036	15.84±0.045	15.93	1.395±0.025	8.83±0.160
S. philippinensis..	18.40± .047	18.36± .006	18.36	1.834± .033	9.97± .181

RATIO OF LENGTH TO DIAMETER

S. spontanea.....	2.71±0.009	2.71±0.011	2.71	0.357±0.006	13.20±0.242
S. philippinensis..	1.91± .007	1.89± .008	1.85	.266± .005	13.92± .256

An examination of the data shows clearly that the long-spored Visayan form, *Sclerospora spontanea*, at least in regard to its conidia, is quite distinct from *S. philippinensis*. The location of the two frequency curves shows that the great bulk of the conidia of *S. philippinensis* fall between the limits of 31 to 36.9 μ in length, and 17 to 18.9 μ in width; while, on the contrary, a like proportion of those of *S. spontanea* are 37 to 46.9 μ in length and 15 to 16.9 μ in width. The somewhat irregular character

of the length curve of the latter species does not, in the opinion of the writer, indicate that it is bimodal, because, by using more inclusive measurement classes of 4 μ or even 3 μ, the depression so noticeable with the 2-μ classes smooths out and the curve becomes quite regular. Moreover, the difference between the modes as well as between the means and the medians is still sufficiently great to emphasize strikingly the dissimilarity in size of the conidia of the two species.

It should be noted that, although the curves of frequency distribution of the two species overlap slightly, size is none the less a valuable diagnostic criterion. In length, for instance, the curves overlap from 26 μ, the lowest limit of the Visayan Sclerospora, to 52 μ, the highest limit reached by *Sclerospora philippinensis*. As a result, it might be contended that size is of no value in distinguishing between the two species when applied at least to the conidia falling between these limits. While this is true of any one conidium, experience shows that, if several are measured, exceedingly few are to be found in this disputed region. For practical purposes even 50 unselected conidia of each species are sufficient to show the difference between them without any confusion due to overlapping.

It is also worthy of note that the curves of the frequency distribution of 700 conidia in both the Visayan species and *Sclerospora philippinensis* differ in no essential particular from those of 500, 400, or even as few as 200 conidia.

Furthermore, in the ratios of length to width of their conidia, the two species also show marked differences. The shorter, broader spores of *Sclerospora philippinensis* most commonly show ratios of 1.55 to 2.14, while in *S. spontanea* the greater length as well as the lesser width of the conidia is expressed by the predominant ratios of 2.35 to 2.94.

In order to determine whether the differences between the biometric characteristics of the two forms were indeed significant, the method quoted by Rosenbaum (*11*) from Reitz and Smith was employed. This method, which compares the difference between the mean or other constants with the probable error of the difference, shows that in *Sclerospora philippinensis* and *S. spontanea* these differences without doubt are significant and can not be the result of mere random sampling. This significance is clearly brought out in Table III.

TABLE III.—*Difference in means of Sclerospora spontanea and Sclerospora philippinensis compared to the probable errors*

Difference in means.			Difference in means divided by probable error of difference.		
Length.	Diameter.	Length over diameter.	Length.	Diameter.	Length over diameter.
μ.	μ.				
7.55±0.183	2.61±0.058	0.798±0.011	41.27	44.96	70.39

The identity of the long-spored, Visayan Sclerospora, then, is clearly established as quite distinct from *Sclerospora philippinensis*. Whether this distinction is sufficient to entitle the former to specific rank depends somewhat upon the judgment of the investigator. The matter could be settled with greater finality if the two fungi were to be grown in pure culture and compared in morphological and physiological details under the controlled conditions of the laboratory, but unfortunately all attempts to grow the two forms artificially have been unsuccessful. In view, however, of such well-defined, although somewhat relative, morphological differences in the conidiophores as the peculiarities of the basal cell and the branch system, and the well-marked and easily measurable differences in size and shape between the conidia of the two fungi, and in view of the constancy and persistence of these points of dissimilarity over a wide range of hosts, through several generations of maize and during three months' cultivation, the writer regards the Visayan form as worthy of specific distinction from *S. philippinensis*. The species, therefore, is described as new, and as it was first found occurring spontaneously on a wild host, it is named *S. spontanea*.

DIAGNOSIS

Sclerospora spontanea, n. sp.

Symptoms, effect on the individual host, and destructiveness to the maize crop as a whole, as previously described by the writer for *Sclerospora philippinensis* (*12*).

Mycelial hyphae and haustoria as described for *Sclerospora philippinensis;* but the clavate hyphae (conidiophore initials) which emerge from the stomata are longer, more slender, and more irregular.

Conidiophores as in *Sclerospora philippinensis*, erect, single or grouped, developing only at night and in dew; comprising basal cell, main axis, more or less complex dichotomous branching system, and terminal sterigmata; but differing in general in greater total length (350 to 550 μ) and more expanded top, and in particular as follows: Basal cell less knobbed and expanded at the base, more slender (least diameter about 5 to 8 μ), and longer (140 to 260 μ), usually exceeding or at least equaling in length the extent of the main axis from the septum to the primary branches. Main axis usually expanding more abruptly above the septum to a greater width (22 to 32 μ) and constricting noticeably (to about 20 μ) below the branches. Branches longer, more slender, less constricted at point of origin, less recurved and ascending, but standing out more stiffly. Sterigmata longer (13 μ), more slender, and straighter.

Conidia resembling those of *Sclerospora philippinensis* in hyaline, finely granular content, thin wall, rounded apex lacking papilla, and rounded base with apiculus of attachment, and in invariable germination by tubes; but differing as follows: In shape, longer and more slender, usually very elongately ellipsoid or cylindrical; in size, showing greater length and less width, the majority being 39 to 45 μ long by 15 to 17 μ in diameter.

Oospores not yet encountered on maize, although an oogonial stage on *Saccharum spontaneum* may prove to be connected.

HABITAT.—Found in the Visayan group of the Philippine Islands principally on cultivated maize (*Zea mays* L.), rarely on the wild grass bugang̃ (*Saccharum spontaneum* L.), and once on cultivated sugar cane (*Saccharum officinarum* L.). Inoculated successfully upon the first two of these hosts and also upon teosinte (*Euchlaena luxurians* Schrad.), and the wild grass *Miscanthus japonicus* (Thunb.) Anders. Extremely destructive to maize, but much less so to the other hosts.

Material of the type will be found in the pathologic collections of the Bureau of Plant Industry, Washington, D. C., and in the herbarium of the Bureau of Science, Manila, P. I.

DISCUSSION

RELATIONSHIP

The two Sclerosporas, *Sclerospora spontanea* and *S. philippinensis*, are undoubtedly closely allied to each other. It is even possible that future investigation will bring to light forms intermediate between them. Such may be the downy mildew on maize seen by Prof. Reinking in the Cotabato Valley and by Gov. Coverston in Lanao Province, both of which places are in the southern Island of Mindanao. On the other hand, the Mindanao form may be as different from *S. spontanea* and *S. philippinensis* as these have proved to be from each other. The writer feels confident that on further search additional Sclerosporas will be encountered in the Philippines both on cultivated hosts and on wild grasses.

The relationship of the Philippine downy mildew Sclerospora to the similar forms described on maize and related crops from other oriental countries has been discussed in connection with *Sclerospora philippinensis* (*12*). Unfortunately the matter can not be settled finally with the data available. As the writer's discovery that suitable material can be secured only at night is very recent, previous publications present measurements and other data inadequate for comparison with living material. In so far as one can judge, however, *S. spontanea*, on account of its longer, more slender spores, is even more sharply distinguished than is *S. philippinensis* from the Javan species, *S. javanica* Palm (*10*), from the species of British India, *S. maydis* (Rac.) Butl. (*1*), and from the normal, short-spored type of the Formosan species, *S. sacchari* Miyake (*9*). It is of interest to note, however, that in the greater length of its conidia, the very character wherein it differs so distinctly from these other oriental species, *S. spontanea* tends to resemble the two abnormally long-spored forms recorded by Japanese investigators. In his account of *S. graminicola*, Ideta (*8, p. 143–145*), in addition to conidia of the size characteristic of the species, mentions a class of conidia having the—

shape of a long ellipse, 38.4 to 57.6 μ long by 19.2 to 24 μ wide.

Also, Miyake (*9*), in his account of *S. sacchari*, describes conidia not only of the usual shape and size, but also of an unusual type—

long ovate, 49 to 54 μ by 19 to 23 μ.

The descriptions and drawings of both these long types of conidia remind one of the spores of *S. spontanea*, even though the latter are characteristically more slender. It is very probable that the occurrence of these long conidial types in Japan and in Formosa indicates the

existence there of strains or species of Sclerospora as yet unrecognized; but what their relationship and significance may be, future investigation must determine.

The relationship of these two Philippine conidial forms to the oogonial stage characteristic of the genus is as yet unknown. Whether *Sclerospora philippinensis* or *Sclerospora spontanea* is connected with the oogonial stage which is so common on *Saccharum spontaneum* throughout the Philippine Islands is yet to be established. The writer has attempted to germinate the oogonia of the latter and to obtain inoculations with them, but so far he has been unsuccessful. Until the precise connection is definitely established, it is well to be cautious about assuming that the two types of spores are with certainty different phases of the same species. It may be worthy of note that the writer has found, in addition to the oogonia on *Saccharum spontaneum*, similar spores on *Miscanthus japonicus* and on cultivated sugar cane in the mountains of northern Luzon. On all these hosts the oogonia are apparently the same species; and their significance and importance will be discussed by the writer in a later paper.

NONSPECIALIZATION

As the problem now stands, the Philippine maize-mildew presents an interesting situation, since it involves two causal Sclerosporas quite distinct morphologically but practically indistinguishable physiologically both in their effect on, and in their virulence to, a range of hosts. The genus Sclerospora seems, then, to present a marked contrast to the strong specialization of the closely related genus Peronospora. In the latter, the work of Gäumann (*5*, 6, 7) has shown that the species are strongly specialized, being distinct on different hosts. This is true especially in the Rubiaceae (*7*), but also to a marked degree in the Cruciferae (*5*) and the Scrophulariaceae (*6*). The distinction holds both morphologically, in the size and character of the conidiophores and conidia, and also physiologically, in their inability to infect any host species but that from which the spores were derived. Gäumann, therefore, regards it as highly improbable that the same host species would be found to harbor more than one species of Peronospora. In Sclerospora, however, we have the two species, *Sclerospora spontanea* and *S. philippinensis*, morphologically distinct, yet both with equal ease inoculating the same series of hosts, including members not only of the Maydeae but also of the Andropogoneae.

SIGNIFICANCE OF OCCURRENCE

The finding of *Sclerospora spontanea* on a wild gramineous host is of interest. Hitherto in spite of the attention which the destructive oriental Sclerosporas have attracted, no conidial representative of the genus has ever been reported as occurring naturally upon a wild host. It is a question whether the occurrence of *Sclerospora spontanea* on wild

Saccharum in the Visayan Islands should be regarded as throwing light on the problem of the origin of the Philippine downy mildews of maize. In the opinion of the writer this and other facts indicate that the native grasses of the Philippines were the original hosts from which the downy mildews passed and are passing to such very susceptible introduced crops as maize. On the other hand, one should not overlook the possibility that the wild Saccharum clumps might have been infected with the downy mildew from badly diseased maize growing near. In this connection it should be noted that in two cases where *Sclerospora spontanea* was found on wild buganğ grass (*Saccharum spontaneum*) the infected clumps were so far distant and so protected from any downy-mildewed maize that there was little possibility of their having been infected thus. In the other cases the infected buganğ clumps were much older than the mildewed maize adjacent; and, because inoculation experiments have shown that buganğ grass is susceptible only as comparatively young seedlings, there is little doubt that the infection in the grass clump had been carried over in the perennial rootstocks and had not been caught from maize.

Moreover, it is worthy of note, also, that the wild Saccharum is very resistant to the effect of the Sclerospora, while maize is exceedingly unresistant. In contrast to the susceptibility to severe injury already noted in maize, wild Saccharum, even though heavily infected, shows only slight striping of the leaves (Pl. 78, B, C), remains undeformed, and is not materially retarded in development. In spite of the downy mildew the plants continue to grow vegetatively, to produce flowers (Pl. 77, B), and to form, by tillering, dense clumps which by extensive rootstocks persist from season to season, still supporting the active and equally persistent parasite. Because, as a rule, it is the introduced host which is most injured by a disease and the original, native host which is relatively unaffected, the indications are that wild Saccharum and not maize is the original host of *Sclerospora spontanea*.

The finding of *Sclerospora spontanea* on sugar cane is a second point of interest. Because, in Formosa, the closely related species *S. sacchari* Miyake had proved indiscriminately destructive to both sugar cane and maize, the writer, while in the Philippines, made especial effort to discover instances of the transmission of downy mildew from one to the other of these hosts. The single case in Cebu, however, was the only one noted. In this instance the single clump of sugar cane infected with *S. spontanea* was situated at the extreme edge of the field, separated only by a narrow trail from a large planting of badly downy-mildewed maize. Although the whole sugar-cane field was carefully inspected, no other cases of Sclerospora were discovered. It is natural to infer that the sugar-cane plant was infected from the neighboring maize, especially since the two parasites proved to be the same. It is rather surprising, however, that this lone cane plant, of all the thousands examined in scores of different

fields adjacent to or even interplanted with infected maize, should be the only one to succumb.

The matter is still further complicated by the fact that in Formosa Miyake easily obtained the infection of sugar-cane plants grown from cuttings, while in the Philippines the writer was not able to inoculate cutting-grown plants of sugar cane, or even of *Saccharum spontaneum*, although seedlings of this grass were readily infected (Pl. 78, A). Moreover, in Formosa the effect of *Sclerospora sacchari* Miyake on sugar cane is far more destructive than was the effect of *Sclerospora spontanea* on this single cane plant. In the former the elongation and weakening of the shoots and the conspicuous yellowish striping of the leaves are a distinct contrast to the stunting of the shoots and faint, pale green markings of the leaves which characterized the Philippine specimens. Also, although the latter died shortly after being transplanted, this was apparently due to the severe treatment they had received rather than to the destructive character of the Sclerospora. It is possible that *Sclerospora spontanea*, in its essential individuality, is much less virulent to sugar cane than *Sclerospora sacchari*, or it may be that some limiting factor is operative in the Philippines. The work of Fawcett (*4*) indicates that temperature differences may exercise an important limiting effect within a smaller geographic range than from Cebu to Formosa. In any case, although the matter is in need of further study, it can safely be said that in so far as has been observed in the Philippines the production of sugar cane is unaffected by *Sclerospora spontanea* or other conidial Sclerosporas.

SUMMARY

The downy mildew of maize which is extremely destructive in the Philippine Islands has been found to be caused by the Peronosporaceous genus Sclerospora. At first only one species was thought to be involved, and this was described by the writer as *Sclerospora philippinensis*. More recently the problem presented by the Philippine maize-mildew has been still further complicated, since a second causal species of Sclerospora has been found to be concerned also. The foregoing paper describes this species as new (*S. spontanea*) and presents briefly its morphological and physiological characteristics and its importance and relationship.

Sclerospora spontanea, the more recently discovered form, occurs in the Islands of Cebu, Bohol, and Leyte, where it was found on the wild grass *Saccharum spontaneum* L., on sugar cane (*Saccharum officinarum* L.), and on maize (*Zea mays* L.). *Sclerospora philippinensis*, the species first recognized, occurs in the Island of Luzon, where it was found on maize, teosinte (*Euchlaena luxurians* Schrad.), and sorghum (*Andropogon sorghum* [L.] Brot.).

Morphologically, *Sclerospora spontanea* is characterized by the relatively much greater length and slenderness of its conidiophores in general and of its basal cells and conidia in particular. In these respects it differs markedly from *S. philippinensis*, which has shorter, stockier

conidiophores, shorter, thicker basal cells, and shorter, broader conidia. There are, moreover, some minor distinctions between the branch systems and between the sterigmata of the two species.

These differences remain constant for each species and are not influenced by growth on different hosts even through several generations. Both species have been artificially inoculated with equal ease from one to another of the following hosts: Maize, teosinte, *Miscanthus japonicus*, and *Saccharum spontaneum*. Attempts to inoculate sorghum artificially were unsuccessful with both species. Because no seedlings of sugar cane were available, no inoculation with either fungus was attempted. Inoculations on sprouted sugar-cane cuttings were uniformly unsuccessful.

Since the size and shape of the conidia are the most useful criteria of interspecies distinction, they are given in detail. Measurements of 700 conidia of each of the two species were combined into comparative tables and graphs of frequency distribution in an attempt to present the differences between them quantitatively as well as qualitatively.

Although morphologically the two species differ as has been described, yet physiologically, in general effect in the field, in effect on the individual plant, and in virulence to the same wide range of hosts no distinction between them is apparent.

The discovery that two forms are involved complicates the problem presented by the Philippine downy mildew of maize. Because two forms morphologically different but practically indistinguishable in physiologic effect are concerned in the same disease, there appears to be a decided lack of that specialization which characterizes certain other genera of the Peronosporaceae. It seems highly probable that still other forms will be found to be concerned in similar diseases in the Philippine Islands and throughout the Orient.

In addition to these two conidial species with a host range of maize, teosinte, sorghum, sugar cane, *Saccharum spontaneum*, and *Miscanthus japonicus*, the writer has encountered in the Philippines oogonial stages of Sclerospora on *Saccharum spontaneum*, *Saccharum officinarum*, and *M. japonicus*. The oogonia on these three hosts are practically indistinguishable. Whether these oogonial and conidial stages are quite unrelated or are indeed only phases in the development of the same organism remains to be determined.

Sclerospora spontanea, like *S. philippinensis*, is closely related to the other conspicuous conidial Sclerosporas of the Orient: *S. javanica* Palm, of Java; *S. maydis* (Rac.) But., of India; and *S. sacchari* T. Miyake, of Formosa. All these forms are characterized by the predominance of the conidial stage, the absence or great rarity of the oogonia, germination of the conidia by tubes, and the occurrence on maize, sugar cane, and related hosts in the Orient. *S. spontanea*, however, because of its longer, more slender spores is as a species distinguished even more sharply than *S. philippinensis* from these other oriental representatives.

The discovery of *Sclerospora spontanea* on wild *Saccharum spontaneum* is, in so far as the writer is aware, the first record of the occurrence of a conidial Sclerospora on a wild host in the Orient. This occurrence, in connection with other data, seems to the writer to indicate that the wild grasses are the natural hosts of these oriental downy mildews from which they have passed and are passing to susceptible introduced crops such as maize.

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25119°—21——2

PLATE 76[1]

Corner of a native-grown maize plot in the interior uplands of Cebu. At the edge of this field, in which many maize plants were being killed by downy mildew, were occasional clumps of the wild grass (*Saccharum spontaneum* L.) called "bugañ" in the Visayan Islands. One of these clumps, which was severely infected with *Sclerospora spontanea*, is shown at the left. The older, primary stalk of this clump, had died, but although the remaining shoots were apparently uninjured, great numbers of conidiophores were being produced on them, especially on the one held out for inspection. The base of this shoot was a few feet farther down the steep slope at the point indicated by the arrow. Behind the central figure can be seen a maize plant noticeably discolored by the downy mildew.

[1] Photographs by W. H. Weston.




A


B

PLATE 77

A.—Clump of *Saccharum spontaneum*, showing characteristic size and habit of healthy plants under natural conditions. The measure is 2 meters tall.

B.—Clump of *Saccharum spontaneum* infected with *Sclerospora spontanea*. When transplanted to this container in Cebu the infected plant comprised a single shoot separated from the clump shown in the preceding plate. This shoot continued to develop vigorously in spite of the downy mildew until after 5½ months it had produced the thriving clump shown. Conidiophores were still being produced in abundance, especially by the younger stalks. Same measure as in A.

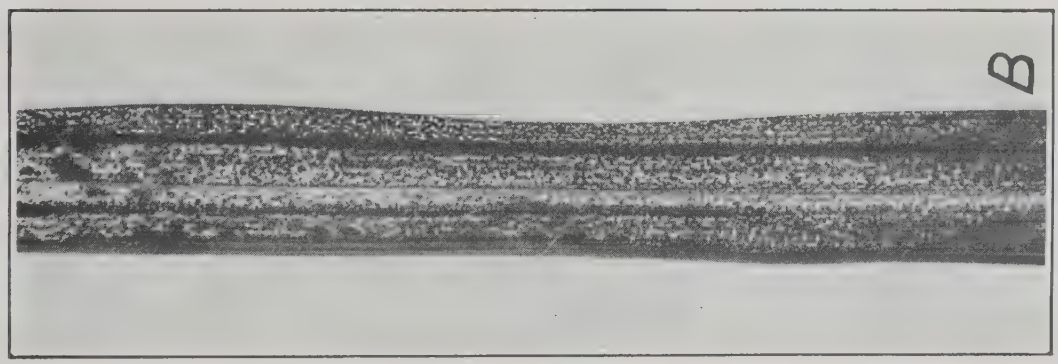
PLATE 78

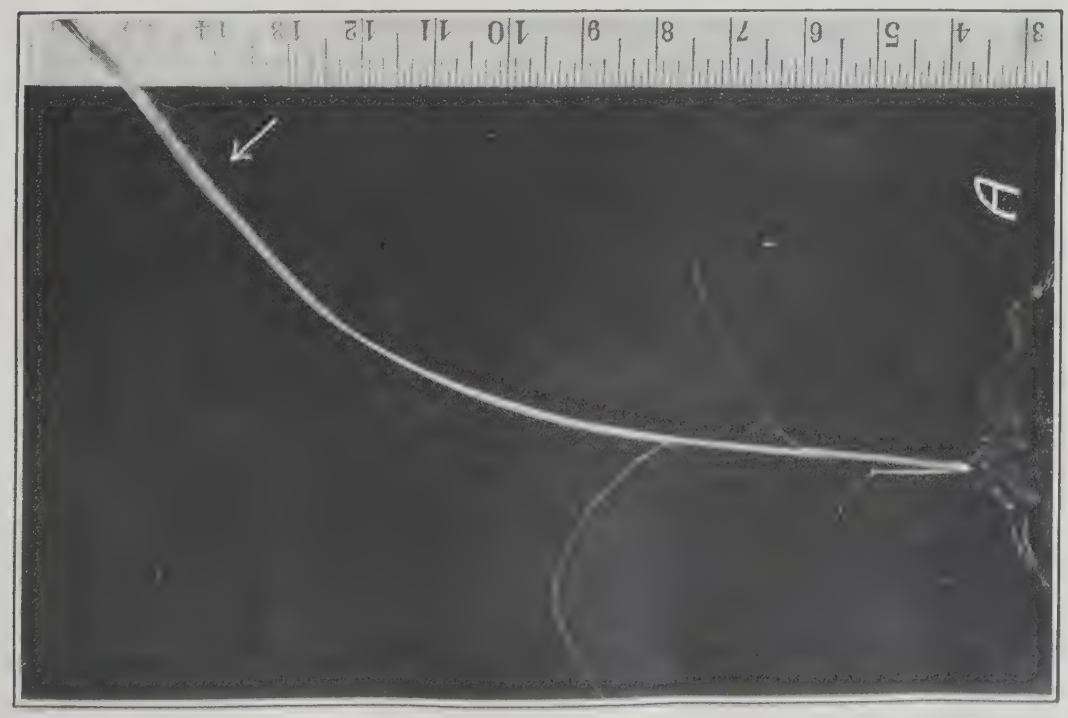
A.—A young seedling (3 weeks old) of *Saccharum spontaneum* infected with *Sclerospora spontanea*. On this seedling, which was artificially inoculated on the second night after it emerged, conidium production began on the sixth night following and recurred in increasing abundance on successive nights. In contrast to healthy seedlings this plant betrays the effect of the Sclerospora in its pallor and in the presence of a whitish "down" of conidiophores. These have collapsed on drying but can still be seen on that part of the fourth leaf indicated by the pointer. × ¾.

B.—Conidiophores on the leaf of *Saccharum spontaneum*. A portion of the upper leaf surface of a downy-mildewed plant (Pl. 77, B) showing remains of the whitish "down" of innumerable conidiophores produced during the night. Although photographed as early as light would permit, the leaf surface has dried somewhat and the fragile conidiophores have shrunk and matted together. × 1½.

C.—Young shoots of *Saccharum spontaneum* arising after the primary stalk had been cut, and like it severely infected with *Sclerospora spontanea*. The main plant, one of the four downy-mildewed ones transplanted from Cebu, was cut off close to the ground. All the subsequent shoots arising from the remaining base were, from the first leaf, badly infected with Sclerospora and produced abundant conidiophores.


C


B


A

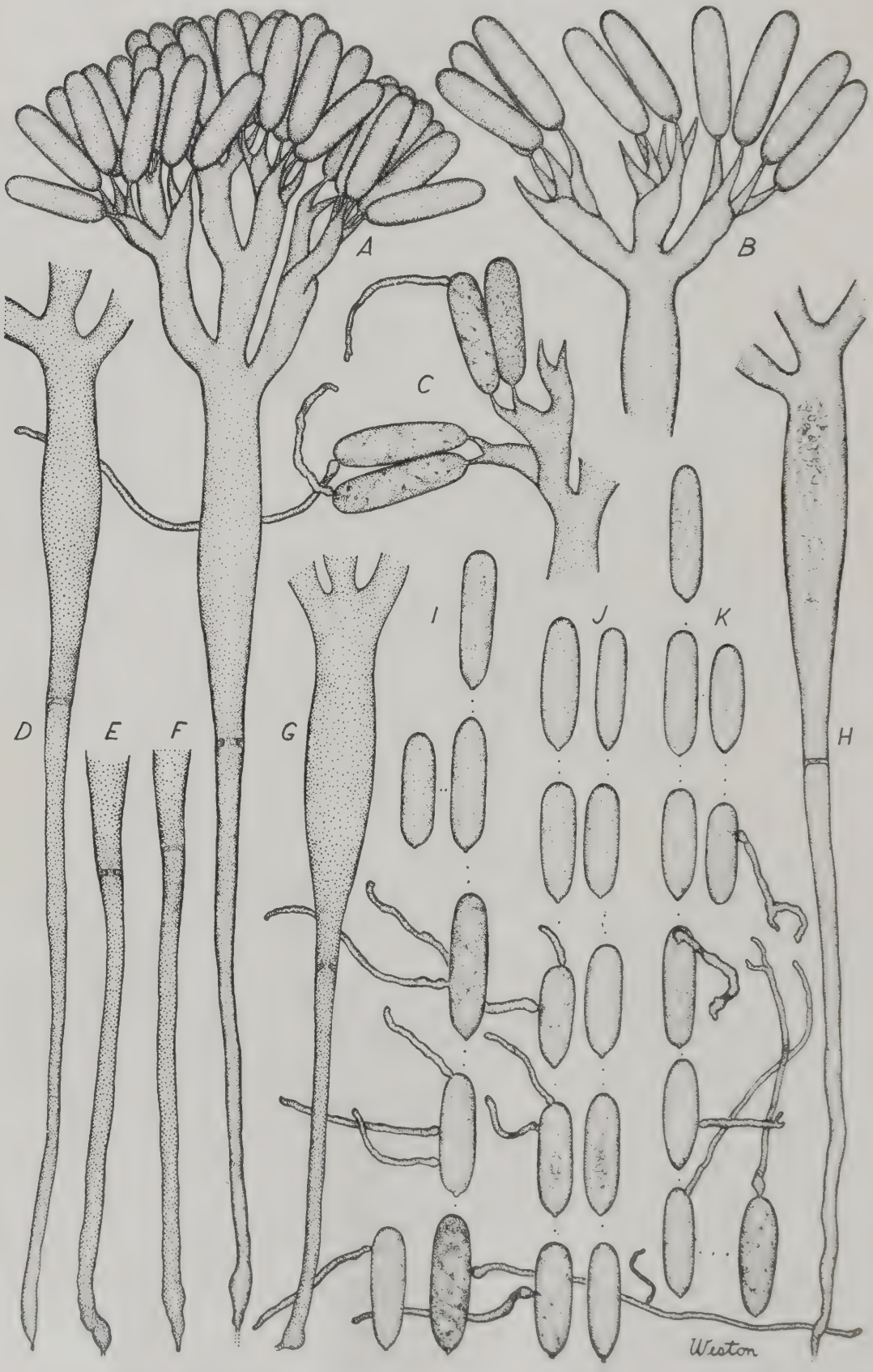

A
B
C
D
E
F
G
H
I
J
K
Weston

PLATE 79 [1]

A.—Typical conidiophore,[2] showing characteristically long, slender, unknobbed basal cell, relatively short main axis with its greatest diameter about midway to the primary branches, and fairly well-developed branch system bearing long, slender conidia. The number of conidia is somewhat less than that usually encountered. From maize inoculated from *Saccharum spontaneum*. × 375.

B.—Upper portion of a conidiophore which has a poorly developed branch system and hence bears few conidia on sterigmata which are relatively large. Several conidia have been broken off in mounting. From maize. × 375.

C.—Portion of the branch system of a conidiophore, showing the conidia germinating while still attached to their sterigmata. From maize. × 375.

D.—Stalk portion of a typical conidiophore, showing long, slender, unknobbed basal cell, and main axis which is slender above the septum, expands rapidly to its greatest diameter about midway, and contracts again below the branches. From *Saccharum spontaneum*. × 375.

E, F.—Typical basal cells of conidiophores. E from *Saccharum spontaneum;* F from sugar cane. × 375.

G.—Stalk portion of a conidiophore with basal cell which, though unusually short, nevertheless is longer than the extent of the main axis from septum to primary branches. From *Saccharum spontaneum*. × 375.

H.—Typical stalk portion of a conidiophore from sugar cane. Compare with A and D. × 375.

I, J, K.—Typical conidia showing variations in size and shape and method of germination by hyphae. I from maize, the lowest figure from material especially fixed and stained to bring out the internal structure; J from *Saccharum spontaneum;* K from sugar cane. × 375.

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[1] The drawings were made with the aid of a camera lucida. Figure A and the ungerminated conidia of figures I, J, and K are from fresh material. All the other drawings are from preserved specimens.

[2] In comparing these drawings with the plates of *Sclerospora philippinensis* (12) it should be noted that the latter give a somewhat misleading impression of the relative spreading of the branch system because the conidiophores were flattened slightly in mounting.

ONION SMUDGE

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INTRODUCTION

Smudge is a common disease of onions occurring both in the field and in storage or transit. It is confined for the most part to the bulbs and is characterized by dark green to black spots of variable size and shape on the outer scales. The spots may be homogeneous in appearance or may consist of numerous individual stromata scattered miscellaneously or arranged in concentric rings. The disease is most common on the white varieties of onions and damages materially the appearance and market value of the crop. The causal fungus has heretofore generally been known as *Vermicularia circinans* Berkeley, but as explained later in this paper it should more properly be termed *Colletotrichum circinans* (Berk.) Voglino.

The present investigations have been carried on with special reference to the disease as it occurs in the districts of southeastern Wisconsin and northeastern Illinois where onion sets are grown. The growing of white onion "bottom sets" is an industry of considerable importance in these sections, and the methods used in growing and handling the set crop are often conducive to the excessive development of smudge during and immediately following harvest. In this study attention has been given primarily to the mycological and physiological aspects of the causal organism, the relation of the parasite to the host tissue, the life history of the fungus with relation to the production of disease, and the development of remedial measures.

THE DISEASE

COMMON NAMES

A number of common names have been used in American and European literature for this disease—namely, "onion Vermicularia" (*3*)[2], "Vermiculariose" (*29*), "black spot" (*7*, *30*), "scab" (*17*, 21), "anthracnose" (*7*, *36*, *37*, *38*), and "smudge" (*26*). The name "anthracnose"

[1] This study was begun in the Department of Plant Pathology at the University of Wisconsin in 1914, and the major portion was completed in 1917. Since the writer entered the Office of Cotton, Truck, and Forage Crop Disease Investigations in the latter year, observations have been extended to sections outside of Wisconsin. Grateful acknowledgments are expressed to Dr. L. R. Jones, under whose immediate direction the work has been done, and to Drs. J. J. Davis and E. M. Gilbert, who have given valuable aid and suggestions on the mycological phases of the problem.

[2] Reference is made by number (italic) to "Literature cited," p. 719–721.

has been much used up to the present time. However, since the symptoms have little in common with those of the more common anthracnoses, and since it is believed that as simple and as descriptive a name as possible should be chosen, the name "onion smudge" is used in this paper to designate the disease, and this name is recommended for general usage.

HOST PLANTS

White varieties of the onion (*Allium cepa*) are the chief ones affected by smudge, but all varieties thoroughly tested have been found susceptible to at least a slight degree. The disease also occurs on shallots (*A. ascalonicum*) and on leek (*A. porrum*). It has never been found on garlic (*A. sativum*).

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Onion smudge was first described in 1851 by Berkeley (*4*) in England, where it was found on the outer scales of a white variety. Subsequent reports of its occurrence in Europe have been made by Massee (*17*) in England, Bubák (*8*) in Bohemia, and Voglino (*35*) and Allescher (*1*) in Italy.

The first collection of this disease in America, made by Michener, was reported by Berkeley (*5*) in 1874. Since that time it has been recorded in literature as occurring in Rhode Island (*3*), Connecticut (*10, 19, 33*), New York (*20, 22*), New Jersey (*13, 25*), Ohio (*26*), Indiana (*21, 34*), Illinois (*30*), Wisconsin (*23*), and Alabama (*2*). Additional data furnished by the Plant Disease Survey show that it has been present also in Massachusetts, Pennsylvania, Delaware, Maryland, Virginia, Georgia, Louisiana, Texas, Minnesota, and Iowa.

It is thus a disease of widespread occurrence; and, indeed, when one considers the fact that thousands of bushels of infected "bottom" sets are being shipped annually to all parts of the country and abroad, it is reasonable to suppose that its distribution is even more general than this summary indicates.

DESCRIPTION OF SMUDGE (PL. 80, 81)

The disease is confined entirely to the scales and the lower portions of the unthickened leaves which constitute the neck of the bulb. It first becomes manifest upon the appearance of minute stromata which form just beneath the cuticle of the host. These are dark green at first, becoming black with age. Depending on conditions of infection, the individual stromata may be scattered miscellaneously over the surface of the bulb, or, as is more commonly the case, they may be congregated in smudgy spots around a few centers of infection. These spots are usually roughly circular and variable in size. They often coalesce and occasionally contain stromata arranged in concentric rings. Under moist conditions the stromata bear acervuli which contain prominent setae readily distinguished with a lens of low magnification. Cream-colored spore masses frequently form on these fruiting bodies.

Penetration of underlying dry scales by the fungus causes similar spots, which are commonly surrounded by yellowish borders. On the fleshy scales the disease first appears as minute, sunken, yellowish spots which gradually enlarge and often coalesce. As the disease progresses, the black stroma of the fungus usually appears; and, with the collapse of the host cells, spots very similar to those on the dry outer scales result. When the dark-colored stroma does not develop before the scale has entirely dried down, the affected portions appear as slightly raised, yellowish spots, giving to white onion sets an unnatural color which is almost as detrimental to their market value as the black, smudgy spots.

The disease makes its appearance early in July under Wisconsin conditions, the fungus living on the outer dead scales and increasing in amount up to harvest time, when the outer two or three scales may be affected. From this time on it penetrates farther into the bulbs, progress depending upon environmental conditions. Badly diseased bulbs tend to sprout prematurely in storage. In most severe cases the fungus penetrates the entire bulb and causes a complete collapse of the fleshy scales.

The foregoing description applies to the disease as it appears on white onions. On colored varieties (red, yellow, and brown) the fungus is confined, with rare exceptions, to the neck of the bulbs where there is little or no pigment in the tissue, and the symptoms in these cases resemble closely those on the corresponding parts of the white varieties.

On shallots the disease appears as smudgy spots very similar to those on onion and is confined to the outer leaves or scales. On leeks similar symptoms prevail.

OTHER DISEASES LIKELY TO BE CONFUSED WITH SMUDGE

Onion bulbs as they mature are subject to attack by a number of fungi which develop saprophytically on the dead outer scales and produce symptoms which may easily be confused with those of smudge. The most common of these are two species of Macrosporium (*Macrosporium porri* Ell. and *M. parasiticum* Thüm.) (*33*), and a species of Phoma, probably *Phoma alliicola* Sacc. and Roum. (*24*). The Macrosporiums produce irregular, dark green spots which are due to ramification of the mycelium through the dead scales, but which lack the stromata and more or less regular outline of the smudge spot. In a moist atmosphere the fungi fruit and develop a dark green mold due to the production of conidia (Pl. 81, F, G). In rare instances black perithecia of *M. parasiticum* are found on the outer bulb scales. Phoma produces small black pycnidia which are often difficult to distinguish macroscopically from the stromata of the smudge fungus. It is commonly associated with *M. porri* (Pl. 81, H). These two fungi commonly attack both white and colored varieties, and in the latter case the pigment in the outer scales is usually destroyed, giving a symptom which is known in the trade as "onion blotch."

Onion smut is sometimes confused with smudge, especially when the former occurs on mature bulbs. In such instances, however, smut usually causes slightly raised, linear lesions which on colored varieties are commonly accompanied by more or less destruction of pigment. The exposure of the powdery spore mass upon breaking of the lesion establishes the identity of the smut fungus.

ECONOMIC IMPORTANCE

The importance of smudge as a detriment to the onion crop may properly be considered from three standpoints—(1) that of reduction of market value as a result of marred appearance, (2) that of actual shrinkage of the bulbs in storage, due to fungus invasion, and (3) that of increased sprouting of onion sets during storage. Thaxter (*33*) calls attention to the reduction of market value caused by smudge, citing an estimate by one grower of an actual loss of several thousand dollars to his crop in one season on this account. There is little doubt that marked spotting by this disease hampers greatly the disposal of white onions, since they are usually grown at a greater expense than colored varieties for a fancy trade which is prone to discriminate against disfigured stock. Under prolonged storage smudge causes a distinct shrinkage of the bulbs and promotes premature sprouting. These last two factors are not usually of material importance on large bulbs, but they are of much significance with respect to onion sets. The latter are usually harvested in August and September and kept in storage until March. The small bulbs are thus subjected to fungus invasion for several months, and data presented later in this paper show that in badly diseased sets the shrinkage may be doubled by smudge during this period.

Sets which sprout badly during storage are a total loss to the owner, since they will not stand shipping and must be discarded. Much of the sprouting of white sets in storage is due to severe attacks by smudge. Experimental data in support of this statement are given later in this paper.

It will be seen, therefore, that smudge is of greater importance than would be suspected from casual observation. In the Chicago district alone, where approximately 1,000,000 bushels of sets are grown annually, the aggregate loss due to shrinkage in weight and sprouting probably runs into many thousands of dollars.

CAUSAL ORGANISM

MORPHOLOGY

The morphology of the causal organism has previously been discussed by Berkeley (*4*), Thaxter (*33*), Stoneman (*32*), Stevens and True (*30*), and Kempton (*16*).

MYCELIUM.—The mycelium ranges from 2 to 8 microns in width, is septate and branching, varying widely with age as to color and size. It

is at first hyaline with few septa, but later the walls thicken and take on a dark green color, oil droplets become more numerous, and septation is more frequent.

STROMATA.—By close intertwining of the thick-walled mycelial threads, dark green to black stromata, usually only a fraction of a millimeter in diameter and few to several hundred microns thick, are formed beneath the cuticle of the host (fig. 1). On nutrient media these stromata commonly coalesce, forming a black stromateoid layer at the surface of the substrate. This coalescence sometimes occurs on the host, but more often the stromata remain distinct and are connected with one another by threads of the dark-colored mycelium. During protracted storage, or under poorly ventilated conditions, excessive stromatal development may occur (Plate 83, B). Thaxter (*33*) describes large, somewhat flattened sclerotia, "jet black externally and white within,"

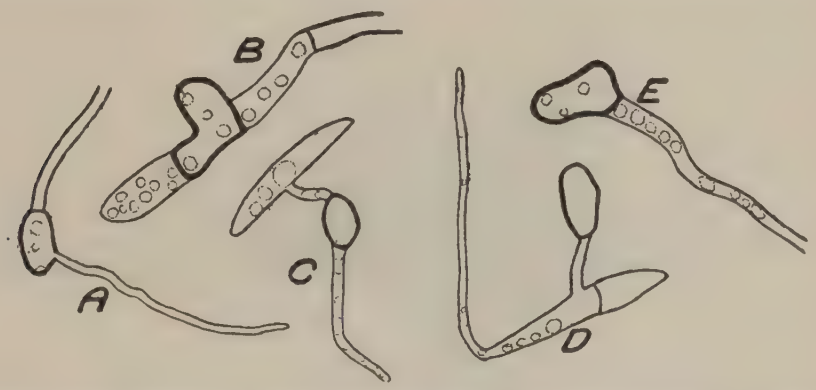


FIG. 1.—Conidia and appressoria of *Colletotrichum circinans*. The fusoid conidia (*C*, *D*) germinate by one or more germ tubes, often becoming septate during the process (*D*). Dark-colored, thick-walled appressoria develop at the tip of the germ tubes, usually as the latter come in contact with the host cuticle (*C*, *D*). Subsequent germination of appressoria commonly occurs (*A*, *C*). Terminal or intercalary appressoria-like cells, or chlamydospores, commonly develop within infected scales (*B*, *E*). Camera-lucida sketch. × 750.

associated with the disease, though he does not definitely state that they are connected with the causal organism. The writer has never found bodies of this sort connected with the disease. On the other hand, sclerotia of *Botrytis* spp., which cause decay of onion bulbs and are commonly associated with smudge, compare favorably with his description.

APPRESSORIA OR CHLAMYDOSPORES.—(Fig. 1). These bodies are variable in size, dark brown in color, thick-walled, egg-shaped or roughly circular, usually terminal but occasionally intercalary. In germination drops on glass slides they form most abundantly where the germ tube comes in contact with the slide and less commonly in the upper region of the drop. Under such conditions they measure 6.5 to 8 microns by 4 to 5.5 microns. In Petri-dish cultures on various types of nutrient agar they are almost invariably produced at the tips of hyphae which come into contact with the glass surface. When "infection drops" containing

viable conidia are placed on the surface of onion bulbs, appressoria or chlamydospores are formed in contact with the scale. Later they send out germ tubes which penetrate the host. They are also commonly found within the tissue of affected scales.

ACERVULI.—The fruiting bodies are formed on the stromata which develop beneath the cuticle of the host. Short, hyaline conidiophores form in a palisade layer and rupture the cuticle of the host (fig. 2). One to several acervuli form on a single stroma. In the study of the morphology of the fruiting body the writer has found no evidence of a closed or partially closed receptacle, as described originally by Berkeley (*4*). Its true nature is more nearly in accord with the work of Stoneman (*32*), who found not a pycnidium but an open fruiting body.

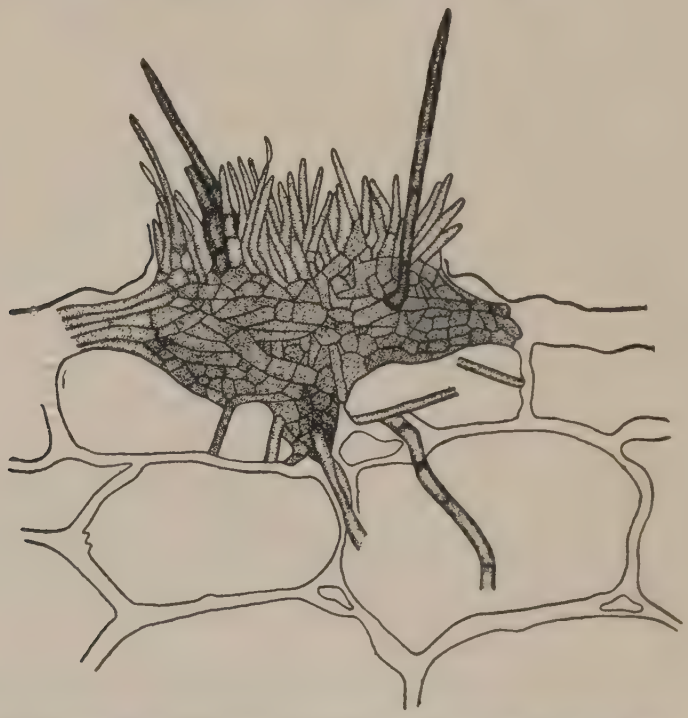


FIG. 2.—Acervulus of *Colletotrichum circinans* on artificially inoculated onion scale. Note the development of the stroma in the subcuticular wall and the rupture of the cuticle by the formation of the palisade layer of the sporiferous hyphae. Camera-lucida outline. × 265.

SETAE.—Scattered throughout the acervulus are numerous setae arising from the basal stroma. They are thick-walled, dark-colored, 0 to 3 septate, upwardly attenuate, and 80 to 315 microns in length.

CONIDIA.—The conidia are borne acrogenously, being budded off one at a time. They are fusiform, continuous, hyaline to slightly ochraceous, somewhat curved, and obtuse at the very apex. Typically one prominent vacuole is present in the center of the conidium, but under some conditions the cytoplasm may contain many large vacuoles. As the spores are budded off from the conidiophores they form a cream-colored, somewhat mucilaginous mass on the top of the fruiting body. The spores vary from 14 to 30 microns in length and from 3 to 6 microns in width. A large majority, however, fall within the limits of 18 to 28 microns by 3 to 4 microns. They germinate usually by one, but occasionally by two or

three germ tubes, which are pushed out at any point on the surface. Septation of the spore commonly occurs during germination.

PERITHECIA.—Stevens and True (*30*) report the development of an ascigerous form on onion sets heavily infected with *Colletotrichum circinans* and have referred the same to the new genus Cleistothecopsis. The writer has never been able to prove *C. circinans* to be connected with any ascigerous form found on onion. Stevens and True claim the connection between the perithecia of Cleistothecopsis and *C.* (*Volutella*) *circinans* on the following evidence:

(1) they occurred on sets badly infected with the Volutella; (2) no other fungi or other types of mycelium were seen to be connected with them; (3) when studied in various stages of development, the typical Volutella mycelium, which offers definite characters for recognition, was seen in organic connection with them, as illustrated in figure 18 (1), (4) the outgrowths from the perithecia are like those of the Volutella.

This evidence is hardly sufficient to prove that the two forms are stages of the same fungus, especially since a large number of saprophytic or semi-saprophytic forms very commonly occur on the dead outer scales of onion bulbs and the differentiation of these from C. *circinans* on the basis of the characters of the mycelium is sometimes very difficult. The writer has, therefore, considered it advisable to use the binomial of the imperfect form until cultures from a single ascus or ascospore of the ascigerous form are shown to be identical with *C. circinans* both as to morphological characters and pathogenicity upon onion bulbs.

TAXONOMY

The taxonomic questions involved in this study concern first, the proper position of the fungus in the present system of classification, and second, the possible identity of the organism with other described species.

Berkeley (*4*) in the original description of the fungus refers to the fruiting body as a perithecium and places it in the genus Vermicularia, giving it the name *Vermicularia circinans*. Thaxter's (*33*) description implies that the fungus has an open fruiting body, but he states that in the early stages of its development a "sort of membrane" extends over the basidia. Miss Stoneman (*32*) describes a thick basal stroma bearing an open fruiting body. She also suggests that the characters of the fungus resemble more closely those of the genera Colletotrichum and Volutella than of Vermicularia. Voglino (*35*), believing the fruiting body to be an acervulus, which would thus place the organism in the order Melanconiales, transferred the species to the genus Colletotrichum. However, he gives no report of any study of the formation of the fruiting body.

Stevens and True (*30*) in discussing the fungus describe a sporodochium consisting—

of a pseudoparenchymatous inner tissue covered by a continuous surface layer... The young sporodochium eventually ruptures its covering membrane... In all cases the conidiophores are borne upon a raised superficial base which constitutes the sporodo-

chium, in contradistinction to the innate form of the acervulus which has no such base. The tubercular swelling, due to the massing of mycelium below and in the epidermis, partakes of sporodochial character also, and while this subepidermal part may not be regarded as constituting a true sporodochium it serves to emphasize the tendency of the fungus to produce such structures... The structure is a tubercle with a differentiated cortical outer layer. This outer layer ruptures and the tubercle develops as a sporodochium... These facts exlcude the fungus from Vermicularia and place it in the Tuberculariaceae under Volutella.

In the discussion later in this paper on the relation of the parasite to the host it is shown that the development of the fungus commonly begins in the outer wall of the epidermal layer of host cells. As the cellulose becomes softened the hyphae multiply and a definite stroma forms within this softened cell wall. Mycelium penetrates the epidermal and underlying cells, and if humid conditions prevail the stroma will soon occupy several layers of subepidermal cells. In good storage this process is comparatively slow, but during a protracted period, especially if the humidity rises considerably from time to time, the stroma commonly does acquire a thickness of several hundred microns. An examination of many sections has shown that regardless of the extent of its development the stroma is always covered by the cuticle of the host. At the instant of sporulation a palisade layer of hyaline hyphae interspersed with dark-colored setae arises from the stroma, and in this process the cuticle is ruptured. This is shown to occur on stromata of widely different ages in figure 1 and Plate 83, B. It is to be noted in the first illustration that the stroma is of recent development, that it is confined to the outer wall of the epidermal layer, and that the cuticle has been ruptured only by the formation of the acervulus. In the second illustration, although the stroma is much greater in extent, the host cuticle is still to be found intact except where it has been ruptured by the two acervuli.

As pointed out by Saccardo (*24, v. 3, p. 221–222, 233*), certain species of Vermicularia are characterized by imperfect or cup-shaped pycnidia, and such forms approach the genus Colletotrichum. Obviously it is often difficult to determine the exact nature of the fruiting bodies, and as a result many forms belonging in Colletotrichum have been placed in Vermicularia. In the form under consideration there is no suggestion of pycnidial development at any time during the development of the fruiting body. On the other hand, it does fall within the limits of the genus Colletotrichum. It is true that the basal stroma is much more highly developed than in many of the better-known species of this genus. However, well-developed stromata have been described in several species of this genus, including *Colletotrichum antirrhini* by Stewart (*31*) and *C. cereale* by Selby and Manns (*27*). In both cases the stroma develops beneath the cuticle, which is ruptured only upon the formation of the acervulus.

It is quite possible that a critical study of the closely related species classified at present in Vermicularia and Colletotrichum will lead to the separation into another genus of those forms which develop acervuli above

thick basal stromata. This question, however, is not within the province of the present paper. Those species of the Hyphales which are placed in the family Tuberculariaceae are characterized by the grouping together of the sporiferous hyphae in a superficial, conglutinate, sessile, or stipitate mass, known as a sporodochium (*24, v. 4, p. 635, 682*). As already pointed out, Stevens and True (*30*) considered the fruiting body of the onion smudge organism to be of this nature and on that basis have transferred it to Volutella. In their description and figures, however, they seem to have interpreted the host cuticle as part of the so-called tubercle and thus as being of fungus origin. Were this true, the stroma would be superficial, and the fungus would properly belong to the genus Volutella. However, since the stroma is always subcuticular and the sporiferous hyphae are subcuticular in origin, the form is more characteristic of Colletotrichum than of Volutella. Here again it is obvious that these two genera need more critical study before their limits can be satisfactorily defined. Meanwhile in the light of evidence just given, the writer considers it more suitable to use the name *Colletotrichum circinans* (Berk.) Voglino for the onion smudge organism.

The comparison of *Colletotrichum circinans* with other related species has been very limited in this investigation. The list of species of this genus which coincide closely with the one in question as to spore measurements and general characters is large and extends over a wide host range. Obviously the comparison of herbarium specimens is insufficient basis for final conclusions under the circumstances. Critical comparison has been confined to *C. fructus* (S. and H.) Sacc., described as causing a fruit rot of apple. This species was originally described as a species of Volutella (*28*), but it was later transferred to Colletotrichum by Saccardo (*24, v. 13, p. 1201*)—

on account of the black setae and the acervulus being originally subcuticular.

Cross sections of apple fruits affected with *C. fructus* and with *C. circinans* are compared in Plate 83, C, D. In both cases the development of the stroma beneath the cuticle, which is ruptured only upon the formation of the acervuli, is clearly shown. The former species was chosen for comparative study because the spore measurements and general characters as previously described were closely similar to those of the onion smudge organism and authentic cultures were available.

Cultures of the apple organism or diseased fruits were secured from Prof. C. R. Orton, State College, Pa., Dr. L. R. Hesler, Ithaca, N. Y., Dr. Charles Brooks, Washington, D. C., and Mr. G. A. Meckstroth, Columbus, Ohio. Cross inoculation on apple and onion showed that *Colletotrichum circinans* was able to produce a rot of apple fruit similar to that produced by *C. fructus* (see Pl. 84, C). The formation of stromata and acervuli by both species on apple is shown in Plate 83, C, D. The rate at which the rot progressed, however, was uniformly slower in *C. circinans*. On onion,

C. fructus developed on the dead outer scale of the bulb, but no evidence of further invasion as occurs with *C. circinans* was observed. Thus, the two species are distinct as to pathogenicity.

Measurement of many hundreds of spores of several strains of both species produced on several substrates including the natural ones—namely, apple and onion—showed that the variations due to differences between strains and substrates along with differences due possibly to slight changes in environmental conditions precluded any distinction on this basis. The slight difference in the shape of spores shown in figure 3 was quite uniform. The spores of *Colletotrichum fructus* have walls nearly parallel throughout the middle half, and one end narrows much more abruptly than the other.

A comparison of growth on potato agar gave further evidence as to the distinction of the two species. The chief points of difference in development on this medium are as follows: (1) *Colletotrichum fructus*

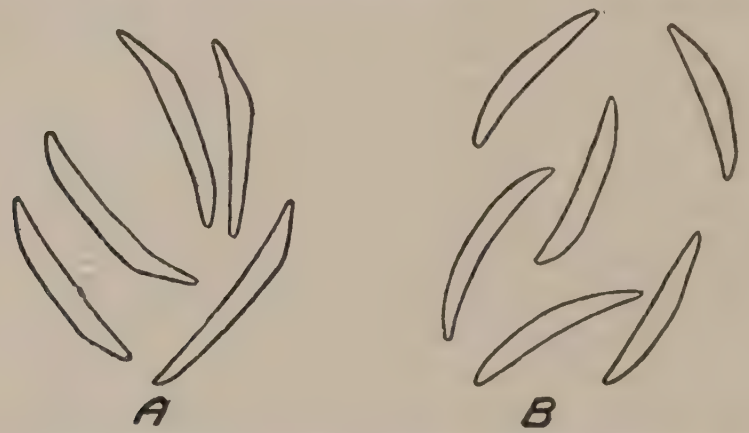


FIG. 3.—Spores of *Colletotrichum fructus* (*A*) and *C. circinans* (*B*). Note the slight difference in shape. In longitudinal section the walls of *C. fructus* are the more nearly parallel throughout the middle half, while at one end they converge more abruptly. Camera-lucida sketch. × 750.

grows the more rapidly, (2) appressoria at the tips of hyphae coming in contact with the glass surface in plate cultures are absent in *C. fructus*, (3) the method of branching is quite distinct—that of *C. circinans* is dichotomous while that of *C. fructus* tends to be monopodial in that nearly straight threads of mycelium, which become dark-colored very early and are greater in diameter, run out radially from the center of the colony and send out hyaline side branches of less diameter. Stromata develop at various points from these radial hyphae. This mode of growth gives a somewhat stellate macroscopic appearance to the colony, which differs from that of *C. circinans*, where distinctly radial hyphae are absent and stromata are scattered. This macroscopic difference is shown in Plate 84.

Thus, although the morphological characters are only slightly variant, the two forms are considered distinct (1) because of difference in pathogenicity, (2) because of difference in spore shape, and (3) because of difference in type of colony on potato agar.

PHYSIOLOGY

ISOLATION OF THE FUNGUS

Pure cultures of the causal organism are readily obtained by the ordinary spore-dilution method. On potato-dextrose agar colonies appear in three to five days. Single spore strains were isolated from such cultures by means of the method described by Keitt (*15*). Isolations thus made from many lots of diseased material collected in Wisconsin, Illinois, Ohio, Connecticut, and Louisiana have yielded strains which are closely similar in their behavior.

CULTURAL CHARACTERS

ON POTATO AGAR (2 PER CENT DEXTROSE) PLATES.—(See Pl. 84,D, E.) The conidium germinates within 6 to 8 hours, sending out one to three hyaline germ tubes, which within 24 hours are many times the length of the spore. Colonies become macroscopic in about 2 days. The mycelium becomes somewhat thicker and denser in the center of the colony, while the younger hyphae around the outer edge are thin-walled and hyaline. Those branches of mycelium which come in contact with glass plates usually produce dark-colored, thick-walled chlamydospores or appressoria. Within 2 or 3 days stromata begin to form by abundant branching from a definite point in the mycelium, which finally results in a thick mass of hyphae. These hyphae assume an olivaceous color, and by the fourth day the dark green stromata are macroscopic in size. They form first at the center and later throughout the colony except at the extreme outer edge. Occasionally they are arranged in such a manner as to give the appearance of "fairy rings," but this is not a constant characteristic. The appressoria and the stromata give the young colony an olivaceous appearance. It becomes darker and almost black with age as the stromata become denser and more numerous and finally form an almost homogeneous stromateoid layer at the surface of the substrate.

By the second day the colony shows a small amount of white aerial mycelium. This increases somewhat with age and later takes on a smoky gray appearance, masking the stromateoid layer to a certain extent. In from three to five days fruiting bodies are formed on the stromata at the center of the colony, and they continue to develop as the colony grows. Conidia are produced in abundance in most strains, accumulating in cream-colored or pinkish masses on the fruiting bodies.

The colony will continue to grow to an indefinite size if space and nutrients are available. A diameter of about 25 mm. is reached in seven days at room temperatures.

ON POTATO AGAR (2 PER CENT DEXTROSE) SLANTS.—Growth is similar in most respects to that on plates. Aerial mycelium tends to be more abundant. Mycelium does not, as a rule, extend deeply into the agar to form stromata. As the culture dries out the aerial mycelium forms a

dense mat over the surface of the culture, its color usually becoming slightly brownish with age. Spore masses often appear above this layer of mycelium.

On other media.—The growth of the fungus was studied on 25 kinds of artificial media, including beef broth agar, corn meal agar, oat agar, apple agar, synthetic agars, vegetable agars, cooked vegetables, and fresh vegetable tissues. The character of growth on the various media used was so uniform and so closely parallel to that on potato agar that a separate description for each is unnecessary. The most noticeable difference was that correlated with the supply of sugar in the medium. Where dextrose was omitted in the formula growth and sporulation were very scanty, and the stromata were few in number and widely scattered. On onion and apple agars made up without dextrose this difference was less marked, probably on account of the presence of a considerable amount of sugar in the plant tissues used. On synthetic agars [1] with sugar added in the form of maltose, dextrose, lactose, and sucrose copious growth took place with no evidence of preference for any one of the carbohydrates used. Cooked bean pod, onion scale, carrot, potato, and rice supported good development of the organism. On fresh onion and apple, however, the growth was much retarded, and on fresh potato and carrot it was very scanty. Stevens and True (*30*) report retarded growth on onion broth agar made with red or yellow varieties. The writer has found equally vigorous development on agar made from red, yellow, and white types of onion.

Relation of Temperature to Growth

Potato agar plates inoculated with mycelium or conidia of the fungus were kept at temperatures ranging from 1° to 35° C. The rate of growth was determined by measuring the diameter of the resulting colonies or thalli from day to day. In order to increase the accuracy of the results Petri dishes of equal diameter containing equal amounts of agar were used. In order to overcome the influence of variations in relative humidity prevailing in different incubators the later experiments were modified by placing the Petri dishes in moist chambers first and then exposing them to the desired temperature. It was found after many trials that the best comparative data could be secured at four to six days. The growth was slight at 1°, almost negligible at 2°, but an appreciable amount occurred at 8° to 10° during a period of 10 to 14 days. Above this point the rate of growth increased rapidly, reaching the optimum at about 26°. At 31° to 32° little or no growth occurred on potato agar. The growth at various temperatures on this medium at the end of 6 days is represented graphically in figure 4.

[1] Formula for synthetic agar used: Sugar, 100 gm.; peptone, 20 gm.; ammonium nitrate, 10 gm.; magnesium sulphate, 2.5 gm.; potassium nitrate, 5 gm.; acid potassium phosphate, 2.5 gm.; calcium chlorid, 0.1 gm.; agar, 20 gm.; neutralized with normal sodium hydroxid.

A similar study of growth in tubes of onion decoction was made, with essentially parallel results. The optimum on this medium appeared to be slightly higher (27° to 29° C.) and slight growth occurred at 31°.

SPORE GERMINATION

RELATION OF MEDIUM.—For the studies upon spore germination a few drops of the liquid medium to be used were placed in Van Tieghem cells. A suspension of conidia in the same liquid was made, and a drop of this was transferred to cover glasses, which were then inverted over the cells and partially sealed with vaseline. The preparations were placed in Petri dishes and exposed to the desired conditions. For some purposes open drops on glass slides placed in Petri dishes lined with moistened filter paper were more suitable.

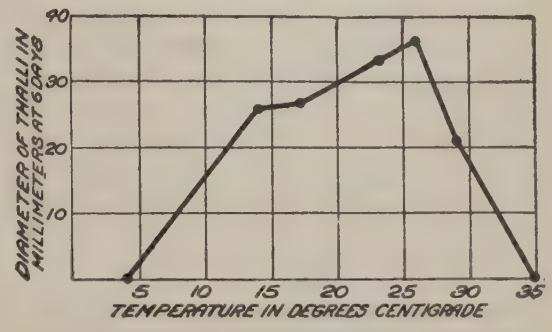


FIG. 4.—Relation of temperature to growth of *Colletotrichum circinans* on agar plates.

A comparative study of spore germination in distilled water, onion decoction,[1] onion leaf extract,[2] onion scale extract,[3] soil extract (sterilized and unsterilized),[4] and soil decoction [5] was made.

At room temperature germination in favorable liquid medium began within 5 to 6 hours. At 24 hours practically all viable spores had germinated. The percentage of germination in the drops was determined by averaging the counts of several microscopic fields. The results of these tests are summarized in Table I.

TABLE I.—*Effect of various media upon spore germination of Colletotrichum circinans*

Medium.	Percentage of germination.
Distilled water	60
Soil decoction	95
Soil extract, sterilized	95
Soil extract, unsterilized	10
Onion decoction	99
Onion leaf extract	0
Onion leaf extract, diluted with distilled water 1 to 10	0
Onion scale extract	0
Onion scale extract, diluted with distilled water 1 to 10	0

[1] Onion decoction: 100 gm. onion scale in 500 cc. distilled water steamed one hour, filtered, and sterilized.

[2] Onion leaf extract: Fresh onion leaves (green) crushed and the sap extracted by squeezing through cheesecloth.

[3] Onion scale extract: Fresh onion scale crushed and the sap extracted as in onion leaf extract.

[4] Soil extract: 500 gm. black loam soil was supported in a glass funnel by excelsior and absorbent cotton; 500 cc. of tap water were poured over the soil; the filtrate was collected twice, and each time it was poured over the soil. The third filtrate was divided into two parts; one part was left unsterilized and the other part was sterilized in tubes at 15 pounds pressure for ½ hour.

[5] Soil decoction: 500 gm. of black loam soil, to which had been added 500 cc. of distilled water, was steamed at 15 pounds pressure for ½ hour. The liquid was filtered through filter paper and sterilized in tubes at 15 pounds pressure for ½ hour.

The striking outcome of this comparison is the marked retardation in unsterilized soil extract and the complete inhibition in onion leaf and onion scale extract. Even when the last two were diluted with 10 parts of water no germination occurred. As pointed out in a previous note by the writer (*38*), further experiments have shown the presence of at least two distinct substances in onion tissue which are probably responsible for inhibition of spore germination. A more detailed study of this phase and its relation to the parasitism of the fungus will be included in another paper. Cooked soil extract, soil decoction, and onion decoction stimulate germination and promote rapid growth of the germ tubes. It is evident that the cooking of the onion scale removes or destroys the substances which are unfavorable for spore germination.

RELATION OF TEMPERATURE.—Since conidia were found to germinate well in distilled water, this medium was used for studies of the effect of temperature on spore germination. A large number of tests were run at a gradation of temperatures ranging from 1° to 35° C. Spores were found to germinate between the limits of 4° and 32°. Appressoria developed in germination drops throughout the same range of temperature. At 35° to 37° slight swelling of the spores took place, giving them the appearance of "involution forms," but normal germination did not occur. Figure 5 is a graphic representation of the effect of temperature as indicated by percentage of conidia germinating in distilled water at 12 hours. Best germination occurred at about 20°, but good germination occurred between 13° and 25°.

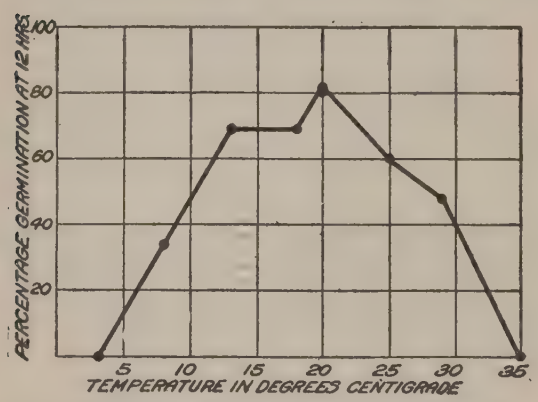


FIG. 5.—Relation of temperature to spore germination of *Colletotrichum circinans*.

The temperature range for spore germination thus coincides closely with that of fungous growth. The point of optimum development is comparatively high, and this fact is significant in explaining the occurrence of the disease in the field.

EFFECT OF DESICCATION

In order to interpret more fully the development of the disease in the field and the overwintering of the causal organism, the effect of desiccation on conidia and stromata was studied in the laboratory.

ON CONIDIA.—Studies were made on conidia as they occur (1) in masses on the fruiting body on the host, where they are embedded in the mucilaginous material which surrounds them, (2) in similar masses on potato agar, and (3) in water suspension, where the spores are separated from one another, approximating to some extent conditions as

they occur in nature when spores are disseminated by meteoric water. Diseased onions bearing spore masses were brought in and allowed to dry out gradually in the laboratory, and the viability of the spores was tested from time to time. Ordinarily a large percentage lost their vitality within 2 weeks, but in some cases good germination occurred after 7 weeks. A small percentage of conidia from spore masses produced on potato agar and exposed to similar conditions germinated after 4 months. Spores in water suspension allowed to dry out on glass slides were very sensitive to desiccation, little or no germination occurring after 24 hours. It is evident, then, that the conidia are sensitive to desiccation except when they remain in waxy masses on the host, in which condition a small percentage will remain viable through extended unfavorable periods. These results are in accord with the findings of Hasselbring (*14*) for the somewhat closely related fungus *Gloeosporium fructigenum*, causing the bitter-rot of apple.

On stromata.—The stromata of the fungus are capable of withstanding very long periods of desiccation. Test tube cultures of the fungus on a large number of media were kept at room temperature for a period of two years. Since the tubes were not plugged very tightly with cotton the cultures dried out completely within four or five months. The vitality of the fungus in this desiccated condition was tested by adding sterile melted potato agar to the tube and slanting them until the fresh medium hardened. Vigorous growth characteristic of the fungus resulted from the cultures originally made on potato, beef broth, carrot, corn meal, oatmeal, and onion agars, steamed rice and bean pods, and fresh potato and onion plugs. The fungus was no longer viable on synthetic agar, steamed potato, carrot, onion, and fresh carrot. Since spores lose their vitality in such a long period of drying, it may be inferred that the fungus lived through this extended period of desiccation by means of the stromata which developed in the substrate. It is to be expected from these results that the stromata which develop in the scales of the host are capable of carrying the fungus over long periods of unfavorable climatic conditions.

Effect of freezing

On conidia.—Spores in water suspension exposed to freezing temperatures are killed within a few hours. Fresh spore masses also are very sensitive to low temperatures, but if they are allowed to dry out before being exposed to freezing temperatures they will withstand such temperatures for a month or more. In order to test the resistance of conidia to the freezing weather of the entire winter period, infected onion bulbs bearing spore masses were placed out of doors in a weather instrument shelter at Madison, Wis., on December 7, 1915. Germination tests showed a high percentage of these conidia to be viable at this

time. Tests made on January 22, 1916, showed that by this date all the spores had been killed. A similar experiment was carried out at Madison in the winter of 1919–20. Infected bulbs bearing abundance of spore masses were placed out of doors in October, 1919, and protected from rain and snow. A few viable spores were obtained on March 20, 1920. Thus, a few conidia may withstand Wisconsin winters if sufficiently protected, but probably few, if any, live over under field conditions.

ON STROMATA.—Agar cultures containing abundant stromateoid development were kept out of doors during the winter months at Madison, Wis., during which period there was much severely cold weather. In all cases the cultures were found to be viable at the end of this time. Stromata on onion scales have also been exposed in this region during the winter period, and in every case they withstood the severe freezing temperatures.

It is to be expected from the foregoing data that spore masses withstand short intervals of dry weather during the summer and furnish ready inoculum upon the return of moist conditions. During extended periods of unfavorable conditions, however, the stromata serve best to perpetuate the fungus.

PATHOGENICITY

Inoculation experiments were performed on plants at various stages of growth from young seedlings to mature bulbs.

Sterilized greenhouse loam soil was inoculated by spraying with a water suspension of spores at the time of sowing onion seed. Three hundred seeds of White Globe variety were planted in the inoculated soil and the same number in uninoculated soil. Ten days later, as the cotyledons were coming through the soil, the attack of the fungus became evident by the rapid collapse of the succulent tissue at any point on the young shoot. Acervuli of the fungus were present and continued to develop on the diseased portions of the plants. Fifteen days after sowing, 64 out of 123 plants in the inoculated pot were diseased, whereas all of the 161 plants in the control pot were healthy. This experiment was repeated several times, and in each case where sterilized soil was inoculated a high percentage of the seedlings were killed. When unsterilized greenhouse soil was used the injury was greatly reduced, the competition of other soil organisms evidently greatly limiting the activity of the smudge fungus. Moreover, damping off of this sort due to smudge has never been noted in old onion set fields, other factors, such as low temperature at this early part of the season, probably limiting the activity of the fungus.

Leaves of half-grown plants were sprayed with a spore suspension and kept in a moist chamber for 24 to 48 hours. The fungus developed and fruited on the lower leaves, which had reached a stage of "physiological old age," but this never occurred on vigorously growing leaves.

The disease was produced many times by means of artificial inoculation of healthy mature onion bulbs with suspensions of spores from pure cultures, and the fungus was readily reisolated. A summary of these inoculations is given in Table II. In certain cases when bulbs kept in a closed chamber were thus inoculated, the experiment was unsuccessful. It was found in such instances that although the spores were capable of germination in water, they did not germinate in the drops on the bulbs. The inhibitive effect of the volatile oil of onion on spore germination was mentioned earlier by the writer (*38*). An accumulation of this substance when several onion bulbs are placed in the small space in a moist chamber may possibly account for this lack of germination. Further studies on this point will be described in a later paper.

More nearly uniform results were secured when sterilized soil was inoculated by spraying with a spore suspension and healthy bulbs then inserted in this medium for a week or 10 days. The outer scales usually became uniformly infected in 7 or 8 days (see Pl. 81, C). When the bulbs were removed and placed in storage, typical invasion of the underlying scales occurred.

TABLE II.—*Summary of inoculation and greenhouse experiments on onion bulbs*

Type of inoculation.	Inoculation No.	Date of inoculation.	Method of inoculation.	Inoculated.			Controls.	
				Number of onions used.	Percentage infected.	Number of days before first note of disease.	Number of onions used.	Percentage infected
In moist chambers.	1,a	July 24	Spray....	5	100	12	0	0
	1	7	...do.....	1	100	6	1	0
	27	Jan. 8	...do.....	5	100	5	5	0
	29	20	...do.....	5	80	13	5	0
	32	20	...do.....	5	0	.....	5	0
	35	July 20	...do.....	4	100	5	1	0
	44	Nov. 21	...do.....	5	100	6	5	0
	49	Dec. 16	...do.....	5	80	18	5	0
	59	Apr. 12	...do.....	3	0	.....	0	0
	61	26	...do.....	2	100	.....	0	0
In soil.....	3	Aug. 26	.....	10	100	.....	10	0
	23	Dec. 3	.....	5	100	8	5	0
	45	Nov. 30	.....	15	100	8	15	0
	50	Dec. 16	.....	9	100	.....	9	0

In general, then, the fungus assumes the rôle of a weak parasite. Actively growing portions of the plant are not attacked except in young seedlings grown under certain conditions. In the field the fungus is confined to the outer leaves or scales, the cells of which are dead or essentially functionless. As the plant approaches maturity the dry outer scales of the bulb are invaded, but the normal fleshy scales are not affected at this time. A few cases have been noted where the fungus

attacked growing scales which were being parasitized by the smut fungus, *Urocystis cepulae*, but apparently a weakening of the plant is necessary before actual invasion of the growing parts occurs. Following harvest there is a gradual invasion of the dormant cells of the fleshy scales of the bulb as previously described. The progress here is usually slow, but in a moist, warm environment there may be a more rapid invasion, resulting in decay of the resting central bud of the onion set.

RELATION OF THE CAUSAL ORGANISM TO THE HOST TISSUE

METHODS

Onion bulbs from which the thin outer scales had been removed were placed in moist chambers. Inoculum consisting of a suspension of spores from pure culture in sterile distilled water was applied to the uninjured surface of the exposed scales, either in drops by means of a platinum loop or as a spray from an atomizer.

For the study of penetration a razor section was cut tangentially from the surface of the scale directly beneath the infection drop so as



FIG. 6.—*Colletotrichum circinans:* Stage of penetration of epidermal cell of onion scale at 66 hours after inoculation. Camera-lucida sketch. Approximately × 430.

to contain the epidermis with a few layers of the immediately underlying cells. This was examined directly *in toto* in a water mount, the absence of chlorophyll in the host cells making clearing and staining unnecessary. For the study of the relation of the fungus to the host tissue following penetration, pieces of inoculated scale as well as of naturally infected fleshy scales were fixed in Fleming's medium fixative, washed, dehydrated, embedded in paraffin, and sectioned according to standard methods of procedure. In some material a satisfactory differentiation of fungus and host was secured by omitting the bleaching of the microtome sections (commonly done after using a fixative containing osmic acid), which left the mycelium black, and then counterstaining the host cell walls with orange G. In other cases the iron haematoxylin and Delafield's haematoxylin stains gave satisfactory results.

PENETRATION

Under optimum conditions germination occurs within 10 hours and appressoria are formed, either sessile or at the end of short germ tubes. Usually the appressorium is flattened to some extent on the side adja-

cent to the cuticle. The penetration tube is formed from the flattened side of the appressorium and penetrates the cuticle directly (fig. 6, 7). Blackman and Welsford (*6*) have pointed out that solution of the host cuticle by invading fungi has never been fully demonstrated; they explain the invasion of bean leaf cuticle by *Botrytis cinerea* as mechanical in nature. The mode of penetration in onion smudge was not definitely ascertained, but it seems highly probable that the germ tube from the adhering appressorium might pierce the thin cuticle by means of mechanical pressure.

SUBSEQUENT DEVELOPMENT

The fungus hyphae, after penetration, develop first between the subepidermal wall and the cuticle, which is rather elastic in nature and can be raised considerably without being ruptured. Figure 6 illustrates the extent of invading germ tubes at 66 hours after inoculation. The nature of the penetration tube and the subsequent development beneath the cuticle are shown in figure 7. In certain other anthracnose fungi—namely, *Colletotrichum lagenarium* as reported by Gardner (*12*), *C. lindemuthianum* by Dey (*11*), and *Gloeosporium fructigenum* by Hasselbring (*14*)—the penetration tube has been described as invading the cell wall directly. This is also the case in *Botrytis cinerea* on bean (*6*), although the germ tube in this instance does sometimes grow horizontally beneath the cuticle. The softening of the subcuticular wall in the case of onion smudge soon becomes apparent by its swelling and taking on a laminate appearance. The hyphae grow through and between the laminae (fig. 8) and by rapid development soon form the beginning of the stroma previously described. The swelling of the outer wall eventually involves the entire lumen of the epidermal cell. Although the greatest amount of fungus growth at this stage takes place just beneath the cuticle, occasional hyphae penetrate underlying cells. As the hyphae attack these cell walls, softening and lamination take place as in the subcuticular wall, while penetration is seemingly accomplished partly by means of chemical action and partly by mechanical pressure. The relation of mycelium to the parenchyma cells just beneath the epidermal layer is also shown in figure 8. In the case of bulbs inoculated in moist chambers the collapse of invaded cells was not rapid, and there was no evidence noted of injury to the cells in advance of the mycelium.

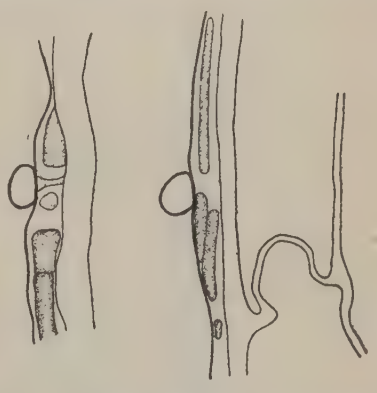


FIG. 7.—Cross section of epidermis, showing early stage of penetration by *Colletotrichum circinans*. Note the empty appressoria with mycelium still wedged between the cuticle and the subcuticular wall. Material fixed 72 hours after inoculation. Camera-lucida sketch. × 700.

Under ordinary storage conditions, the progress of the fungus is closely parallel to that just described, except that the progress is much slower under this different environment. As described before, the first macroscopic symptom of invasion from spots on the dry outer scale to the underlying fleshy scale is a small, yellowish, slightly sunken area. This usually increases in size very slowly in well-ventilated storage. A cross section of one of these spots is illustrated in Plate 83, A, and a detailed drawing from a similar section is shown in figure 9. The fungus develops extensively at first just beneath the cuticle, and the softening and lamination of the subcuticular wall is very slight. As invasion progresses, hyphae penetrate this wall directly, evidently by chemical solution rather than mechanical pressure, since the cavity is slightly larger than the mycelium and there is no sign of bulging of the wall before penetration is achieved. The collapse of cells beneath the epidermal

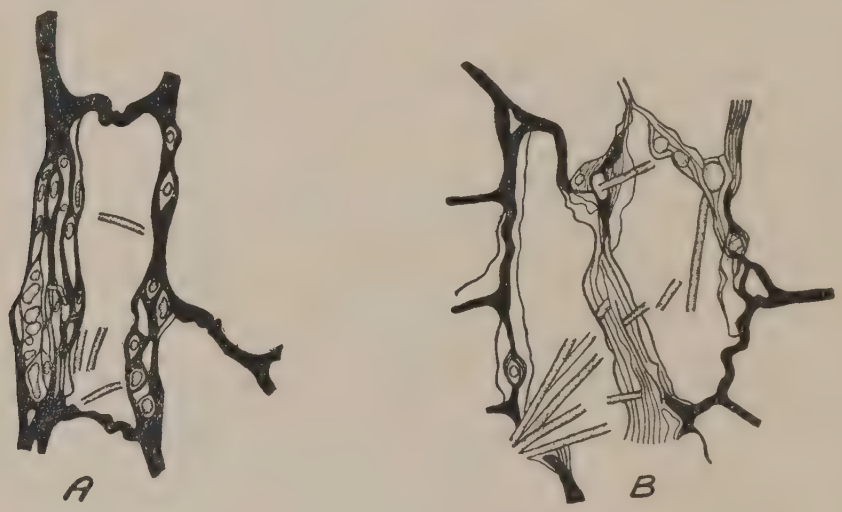


FIG. 8.—Cross section of epidermis (*A*) and underlying parenchyma cells (*B*) of onion scale inoculated with a suspension of *Colletotrichum circinans* spores and kept in a moist chamber at room temperature. Note softening and lamination of cell walls by the invading hyphae. Material fixed five days after inoculation. Camera-lucida sketch. *A*, × 308; *B*, × 350.

cell takes place before any appreciable invasion of hyphae occurs. In the section shown in Plate 83, A, two layers beneath the epidermal layer have collapsed, while only an occasional hypha is to be found beneath the subcuticular wall. There is no evidence of softening of the cell wall. Morever, in such lesions mycelium has never been found in the walls or lumina of turgid living cells. This suggests that either the cells are killed in advance of the hyphae or only slight invasion of the wall leads to their collapse. This slow invasion, which prevails even after the cells have become functionless, is surprising in view of what occurs when bulbs are inoculated in moist chambers. Is it possible that the volatile oil present in the onion scale is influential in checking the advance of the fungus?

Under moist conditions and optimum temperature the stroma develops very rapidly in the subcuticular wall, and acervuli are formed in five to

six days after inoculation. This condition is shown in figure 2. In other cases where sporulation is postponed through lack of proper environment the stroma continues its growth more slowly and eventually involves a larger portion of the scale. The cuticle, however, remains intact on the exterior and normally is not ruptured until the palisade layer of conidiophores is formed. A cross section of a scale which had been held in poorly ventilated storage several months is shown in Plate 83, B. Acervuli were produced upon exposure to proper conditions for sporulation. Note that the cuticle is still present outside the extensive stroma, except where it has been ruptured by the sporiferous hyphae.

FACTORS IN THE PRODUCTION AND PROGRESS OF THE DISEASE

OVERWINTERING OF THE CAUSAL ORGANISM

The experiments already reported on the effect of desiccation and freezing upon conidia indicate only a remote possibility that the fungus lives through the winter in this form under Wisconsin conditions. The stromata, on the other hand, are capable of withstanding protracted periods of drouth or freezing temperature. In order to confirm the supposition that the fungus actually overwinters and is widely disseminated in this latter form, four lots of heavily infected bulbs were placed out of doors at Madison, Wis., on December 7, 1915.. One lot was left in an instrument shelter near the surface of the ground, and the remaining lots were buried in the soil at depths of 2, 4, and 6 inches, respectively. Spore masses were present on this material at the beginning of the experiment, and germination tests showed a high percentage of the conidia to be viable at this time.



FIG. 9.—Cross section of onion scale naturally infected with *Colletotrichum circinans*, showing the mycelium developing first just beneath the cuticle and later penetrating the subcuticular wall. Camera-lucida sketch. × 450. (This phase is illustrated further in Pl. 83, A).

On January 22, 1916, examination of spores from the bulbs placed in the instrument shelter showed that they had completely lost viability by that date. The four lots of bulbs were examined on April 12, 1916. Those which had been buried in soil readily produced conidia in abundance upon exposure to humid conditions at room temperature. The material kept in the instrument shelter had dried out considerably during the winter and, though much slower to respond, eventually proved to be viable by the production of spores. A similar experiment conducted during the winter of 1916–17 yielded confirmatory data.

It is to be expected that infected scales from the crop of the previous season furnish a source of abundant inoculum for initial infection of the growing crop. This, combined with the fact that in most onion-growing sections it is the common practice to grow this crop successively

on the same field for many years, results in a heavy infection of a large part of the white set crop annually. Examination of a large number of fields in Wisconsin and Illinois has revealed the fact that "clean" white sets are secured as a rule only from land growing its first crop of onions. In a majority of cases the second crop of white sets is badly infected.

In all fields examined where the first crop of onions was being grown, an occasional bulb infected with smudge was found. A satisfactory explanation of these original infections has never been reached. Many possible means of introduction of the fungus from neighboring infected fields immediately suggest themselves, such as manure, farm implements, man and farm animals, drainage water, and wind, and undoubtedly some of these often do play a part in the distribution of the disease. The possibility of seed as a carrier is also to be considered in this connection. Although smudge has never been found attacking the floral parts of the plant, it is conceivable that those seed umbels which fall over and come in contact with the soil before harvest might become infected or be the means of introducing bits of infected scales to the seed. It should be noted in this regard that the spores of onion smut, a disease which is also confined to the bulb and leaves of the plant and in fact does not attack onion seed plants, have previously been found on onion seed samples (*9*, *18*).

One experiment was performed on the relation of seed to the dissemination of the fungus. Samples of six varieties of seed were sown in pots of sterilized soil in the greenhouse on December 5, 1916. On January 16, 1917, all the seedlings were examined. Fruiting bodies of *Colletotrichum circinans* were found on the outer scales of two seedlings of the White Globe variety and of one seedling of the Queen variety. No other signs of the disease were found. The identity of the fungus was confirmed by isolation of pure cultures and comparison with authentic strains. Two subsequent plantings of the same sample of White Globe seed were made, but no further sign of the disease was found. The small amount of the fungus occurring in this experiment is not surprising, since only a very limited amount of infectious material can be expected to be seed-borne. However, although the evidence at hand indicates that the fungus is carried on seed to some extent, further data are necessary before a final conclusion on this point can be made.

RELATION OF TEMPERATURE TO INFECTION AND TO DEVELOPMENT OF THE DISEASE

Studies of the relation of temperature to the germination of conidia and to their subsequent growth have shown the optimum to be about 20° C. for the former and 26° for the latter. The range in each case, however, is wide. Accordingly a set of experiments was started for the purpose of determining the range and optimum temperature for infection.

Sterilized loam soil in glass or glazed crock jars was inoculated with a water suspension of spores. Healthy white onion sets were then

inserted in the soil; and the jars, each covered with a glass plate, were placed in incubators running at temperatures ranging from 5° to 32°.

In the first experiment 10 onions were placed in each of four jars which were placed in incubators held at 5°, 13° to 14°, 23°, and 28° to 31° C., respectively. The extent of the disease on the various lots at this time is shown in Plate 82. It was apparent that infection took place very slowly at 13° to 14°, while that at 28° to 31° was slightly less advanced than at 23°.

In the second experiment jars containing 10 onions each were held at 5° to 6°, 9° to 10°, 14° to 15°, 17° to 18°, 20° to 21.5°, 22° to 23°, 26° to 27°, and 30° to 32° C. They were allowed to remain for 17 days before examination. At the end of this period, no infection had taken place at 5° to 6°, a very slight infection at 9° to 10°, and as the temperature rose the amount of disease increased up to 26° to 27°, at which point it was greater than in any of the other jars. At 31° to 32° it was slightly less than at 26° to 27°. A third experiment confirmed the results of the first two.

Infection takes place and the disease progresses, then, at or above 10° C., but it is quite evident that for very rapid development a temperature of 20° or above is needed. Since the fungus develops in the soil prior to infection, the range of soil temperature during the growing season is undoubtedly an important factor in determining the severity of the disease.

PRODUCTION AND DISSEMINATION OF CONIDIA

After the appearance of the first stromata on the bulbs, subsequent spread of the disease is effected to a considerable extent by conidia

Sporulation does not take place except under fairly humid conditions. In order to determine the range of temperature at which fructification may occur, infected scales were placed in Petri dishes lined with moistened filter paper and exposed in incubators running at a range of temperatures from 2° to 28° C. Abundant sporulation occurred within 36 hours at 20° to 28°. The process was much retarded at lower temperatures, though a few spores were formed at 2° to 3° after several days.

Under optimum conditions for spore production the conidia accumulate on top of the acervuli, forming gelatinous masses which remain intact among the setae. Exposure of portions of scales bearing fresh spore masses over sterile agar plates has yielded no indication of spore discharge. The mucilaginous material surrounding the spores appears to dissolve partly when a spore mass is placed in water, and the conidia thus become separated.

It is thus to be expected from the nature of the fungus that warm, rainy weather is especially favorable for the development of smudge, since high humidity promotes the production of spores, and meteoric water, especially in the form of spatt ng rain drops, is important for their dispersion and dissemination.

CORRELATION OF CLIMATIC CONDITIONS WITH THE DEVELOPMENT OF THE DISEASE IN 1915–16

Plots of white onion sets were grown in 1915 and 1916 on land which had previously produced many successive crops of onions and where the smudge organism was known to be present in the soil. Soil temperature records were taken at a depth of 1 to 2 inches during part of the 1915 season and most of the 1916 growing season. The daily mean soil temperatures and rainfall for these seasons are represented in figure

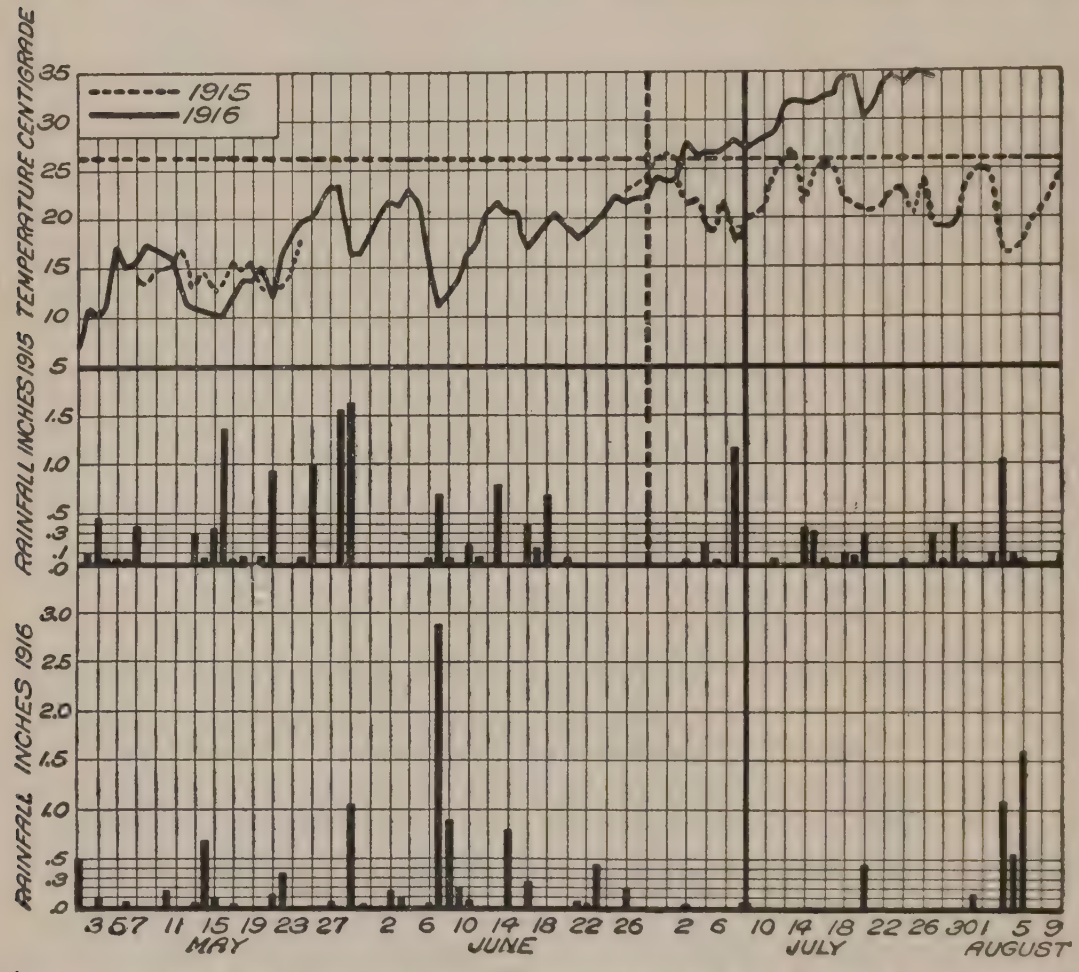


FIG. 10.—Chart from data collected at Racine, Wis., during 1915 and 1916, showing the daily mean soil temperature at a depth of 1 to 2 inches, and the rainfall. The horizontal broken line represents the optimum temperature for infection and development of the disease as indicated by controlled experiments, the broken vertical line the date of first observation of the disease in 1915, and the heavy vertical line the first appearance of the disease in 1916.

10. The rainfall records included here are compiled from data taken at the Racine (Wis.) post office, approximately 3 miles from the onion set plots. The progress of the disease between the time of its first seasonal appearance and harvest is described for these two seasons, since they represent distinctly different conditions which had varying effects upon the progress of the disease.

IN 1915

On June 28 a very few dark green stromata were found, but no acervuli or setae had developed. The soil temperature mean was now well

above 20° C. and remained between 20° and 27° for most of the time until harvest. On July 2 a few scattered acervuli were found. A slight precipitation was recorded on July 2, 2 inches on July 4, 0.02 inch on July 5, and 1.17 inches on July 7. Following this rainy period there was a marked increase in number of acervuli noted on July 10. A slow rain fell during most of July 14 and part of July 15. On July 15 the disease was prevalent above the bulbs on the unthickened portions of the outer leaves which comprise the "neck." These infections were clearly the result of spores spattered upon these portions from the bulb scales by rain a few days previously. The rainy weather, which prevailed until harvest, about August 10, resulted in continued spread and development of the disease, so that the white sets were all badly spotted by the latter date. Further observations showed that the development of the disease in other fields followed closely that noted in the experimental plot. The infection in practically all cases, however, was confined to one or two of the outer dry scales, the fungus being unable to attack the fleshy scales previous to harvest. On the yellow and red varieties the fungus was very abundant on the uncolored portions of the leaves at the neck, but the highly colored bulb scales remained entirely free from it. This has been the usual observation with the colored types.

IN 1916

The month of July, 1916, was extremely warm and dry as contrasted with cool, moist weather of the same period in 1915. The soil temperature mean passed 26° C. on July 2 and remained above that point for the rest of the month. In fact, for a large portion of that period it was well above 32°, the maximum temperature for growth of the fungus on potato agar. No signs of smudge were found until July 8. The extent of the disease at this time was very meager, only a few acervuli being noted. It is probable that the dry weather preceding this date checked the fungus, in spite of the fact that the soil temperature was favorable. Aside from 0.03 inch precipitation on July 8, 0.45 inch on July 20, and 0.14 inch on July 31, no rain fell during the rest of the month. Moreover, the soil temperature was well above the maximum for development of the disease. On July 13 but very little smudge could be found. On July 22 no further development was noted. The moisture from the shower of July 20 disappeared very rapidly from the upper 2 inches of soil because of the extreme heat. A rainy period occurred on August 3, 4, and 5, and following this *Macrosporium porri* and *Phoma alliicola* developed rapidly. Smudge increased but very slowly, however, probably because of the scarcity of viable spores. Another heavy rain fell on August 9 and 10, and the weather then remained clear until after harvest on August 23. At the latter date the bulbs were examined carefully, and in general the sets were only moderately infected. The disease was confined for the most part to the portions of the bulbs below the surface of the soil, while the abundant

infections on the necks which were so conspicuous in 1915 were almost entirely absent.

To summarize, the disease progressed most rapidly during the last part of the growing season of 1915, with the mean temperature range between 20° and 30° C., accompanied by sufficient rainfall to promote abundant spore production and dissemination as well as subsequent infection. On the other hand, development was materially checked in 1916 by extreme heat, together with lack of precipitation during July.

RELATION OF ENVIRONMENT DURING CURING TO THE DISEASE

The onion set crop is usually harvested in early August. The tops are twisted or clipped and the small bulbs are placed in shallow crates 2 or 3 inches deep. These are stacked in the field in piles with temporary roofs, where they are allowed to cure for several weeks. Usually the fungus is well established upon the outer scales of the bulbs before they are pulled, and thus further invasion is dependent largely upon the environmental conditions which prevail during the curing and storage periods.

The respiratory functions of the living cells in the bulbs continue after the sets are pulled, and there is, in consequence, some accumulation of moisture. This is counteracted in part by the use of shallow crates which are exposed to natural air currents. In bright, windy weather the bulbs cure rapidly, while rainy or humid weather retards the process and favors the progress of the disease. A number of experiments were conducted during 1916, 1917, and 1918 to determine the effect of varied amounts of external moisture during the curing period upon the development of the disease.

EXPERIMENT 1.—On August 15, 1916, a crate of white sets was taken from the general run of the crop which had been harvested on August 9 at Racine, Wis. The outer scales were badly spotted with smudge, and in some cases the second scale had been invaded. After removal to the laboratory the bulbs were sprinkled with water while in the crates. After two days a portion of this lot (5½ pounds) was dried for 24 hours at 45° to 52° C. and the remainder (14½ pounds) was given no further treatment. Both lots were placed under cover in a shallow crate, where they were exposed to good conditions for further natural curing. They were later placed in a well-ventilated onion warehouse held at about 35° to 40° F. On January 13, 1917, both lots were examined. Most of the outer dead scales present at harvest time had sloughed off during storage, and in the dried sets the fungus had advanced very little from these original infections. In the naturally cured sets, however, the fungus, probably aided by the greater excess of moisture present, had invaded several underlying scales, and these sets were badly spotted even after the outer scales were removed. The sets in each lot were then sorted into three classes—(1) free from disease, (2) slightly diseased,

(3) badly diseased. The result of this classification is given in Table III, and samples from the dried and the undried lots are shown in Plate 85, A, B.

TABLE III.—*Relation of artificial curing to the development of onion smudge*

Treatment.	Condition at end of storage period.		
	Percentage free from disease.	Percentage slightly diseased.	Percentage badly diseased.
Naturally cured	7	29	64
Artifically dried	56	36	8

EXPERIMENT 2.—On August 30, 1917, several bushels of white onion sets were secured from a field where the crop had been harvested on August 16 and placed in stacks of shallow crates. The weather had been clear during this intervening period, and good natural conditions for curing had prevailed. Smudge was prevalent on the outer scales of the sets at this time. In order to test the effect of exposure to moist weather on the progress of the disease, a portion of this lot in the crates was sprinkled with water daily for one week, approximating roughly what often occurs when a rainy period comes during harvest. After one week a part of the moistened lot was placed in a kiln drier, where the temperature was held at 100° to 120° F., until the bulbs were thoroughly dried. The remainder of this lot was allowed to dry naturally under cover. All the sets were then stored in a standard onion storage house. Samples taken from a moistened and an unmoistened crate on October 10 are shown in Plate 85, C, D. Marked increase in the amount of smudge was very noticeable within a few days after moistening was begun. On January 14, 1917, the amount of smudge was estimated by classifying several hundred bulbs from each of the three lots into either of two classes, namely, (1) those free from smudge or only slightly diseased and (2) those so badly diseased as to impair their market quality. The results are given in Table IV.

TABLE IV.—*Effect of varied conditions at harvest on the amount of smudge on stored onion sets*

Treatment.	Condition at end of storage period.	
	Percentage free from smudge or slightly diseased.	Percentage badly diseased.
Best natural curing	58	42
Exposed to moist conditions after harvest	7	93
Artificially dried after exposure to moist conditions	52	48

This experiment shows (1) that even under what may be considered very good weather conditions for natural curing a considerable amount of smudge will develop; (2) that exposure to moist weather for a week after harvest practically doubled the amount of smudge; and (3) that thorough artificial drying immediately after such exposure counteracts the effect of excessive moisture.

EXPERIMENT 3.—The sets used in this experiment were from a late sowing and consequently were not harvested until September 14, 1918. Smudge was prevalent on the extreme outer scales of a large percentage of the bulbs at this time. Five bushels were placed in shallow crates in the kiln drier, in which the temperature was maintained at 100° to 120° F. One crate was removed at the end of one day, a second at the end of two days, and the remaining three on the fifth day. Three untreated crates used in the experiment were allowed to cure in a covered pile in the field with the remainder of the crop. On September 30 they were removed to a standard onion warehouse, where they were stored during the winter with the artificially dried lots. On March 5, 1919, when final notes were taken, a comparison of the artificially cured and field-cured lots was secured by estimating the percentage showing any signs of smudge after sets had been milled to remove the loose scales.[1] The results are given in Table V.

TABLE V.—*Amount of smudge on artificially cured and field-cured onion sets at the end of the storage period*

Crate No.	Nature of treatment.	Length of treatment.	Percentage showing any signs of smudge.
		Days.	
2	Artificially dried	1	3
3	.....do	3	22
1	.....do	5	21
4	.....do	5	33
5	.....do	5	31
8	Field-cured	16	72
9	.....do	16	75
10	.....do	16	78
	Average of artificially dried crates	.....	22
	Average of field-cured crates	.....	75

The foregoing experiments clearly establish the importance of moisture as a factor in the advance of the disease during the curing and storage periods. They also indicate that artificial curing immediately following harvest greatly checks the progress of the disease as compared with natural field-curing.

[1] It is the common practice to run "bottom" sets through a fanning mill as they are taken from storage in order to remove the loose outer scales.

RELATION OF STORAGE CONDITIONS TO THE DISEASE

The study of the disease in storage has been directed toward the solution of three problems: (1) The importance of smudge as a cause of premature sprouting of sets; (2) the extent of shrinkage, if any, which can be brought about during the storage of onion sets; and (3) the amount of new infection or actual spread from diseased to healthy bulbs occurring during the holding period. While the data on these points are by no means complete and the factors involved in the progress of the disease during the storage period by no means fully studied, the experiments here reported upon throw some light on the matter.

Observations on the first two questions were made in a standard onion set warehouse at Morton Grove, Ill. In practice, onion sets are stored in crates about 4 inches deep with slatted bottoms, piled so as to allow a 1- to 2-inch space between each two crates to facilitate circulation of air. Sets are placed in storage during September and October. The temperature is gradually lowered, following seasonal changes, until it approaches 0° C. (32° F.), an attempt then being made to hold it slightly above this point. During extremely cold weather some artificial heat in the house is necessary to prevent freezing, while ventilation is constantly needed to remove excessive moisture.

The experiments were carried on during the winter of 1918–19. The extremely mild weather during this season prevented the temperature of the house from being held as close to 0° C. as is commonly the case, while, on the other hand, ample opportunity for ventilation was afforded. Continuous records of temperature and relative humidity were secured by means of a Friez hygro-thermograph. The temperature gradually lowered during October and November, the minimum temperature reaching 0.5° C. (33° F.), on November 23, while the maximum temperature commonly reached 12.7° C. (55° F.) during this period. During December, January, and February the temperature fluctuated between 0.5° and 7.2° C. (33° and 45° F.). The relative humidity varied between 65 per cent and 85 per cent during October and November, while throughout the remainder of the period it seldom went above 75 per cent and not often below 60 per cent.

RELATION OF SMUDGE TO SPROUTING

Two lots of onions were used in these experiments, and, since they differed somewhat as to time of maturity and method of handling, they are here considered separately.

EXPERIMENT 1.—Bulbs averaging about 1 inch in diameter were selected from a lot of white sets harvested early in August and brought into storage on August 22, 1918. Two groups were secured, one consisting of 49 bulbs badly spotted with smudge and the other containing 47 perfectly healthy sets. The two lots had thus been grown and handled alike and presumably differed only as to infection with smudge.

They were carried through storage and examined on February 18, 1919. The results are given in Table VI.

TABLE VI.—*Relation of smudge to sprouting of onion sets in storage*

EXPERIMENT 1

Condition of bulbs.	Total number of bulbs used.	Number sprouted.	Percentage sprouted.
Healthy	47	14	29.7
Diseased	49	26	53.0

EXPERIMENT 2.—The sets used in this experiment were sown late in the spring and consequently were not harvested until about September 14, 1918. They were allowed to cure in the field in the normal manner until September 30, when they were placed in storage. Three average crates were selected at this time and kept under observation. At harvest time smudge was prevalent only on the dry outer scales of the sets, but during the storage period it gradually penetrated the underlying scales. When a final examination was made on March 5, 1919, it was clear that in nearly every case where the fungus had penetrated deeply the bulb had sprouted and had thus become worthless. A typical example of this condition is shown in Plate 81, D. An estimate of the amount of sprouting actually due to or intimately associated with smudge was secured by counting 100 to 200 bulbs in each crate. The results are given in Table VII.

TABLE VII.—*Relation of smudge to sprouting of onion sets in storage*

EXPERIMENT 2

Crate No.	Number of bulbs examined.	Total percentage infected by smudge.	Total percentage sprouted.	Total percentage sprouted and showing advanced stage of smudge.
1	165	75	6.0	6.0
2	197	75	9.6	9.6
3	148	72	2.0	.7
Average		74	5.8	5.4

It is not to be construed from these data that smudge is always the chief cause of premature sprouting of onion sets in storage, since unquestionably other factors may often be entirely responsible. One of these, the neckrot decay of the scales, commonly produces a similar effect. It is apparent, however, that the invasion of the bulb scales by the smudge fungus brings about some physiological change which promotes growth of the previously dormant bud.

Economically this factor has considerable value, since bulbs which sprout before the end of the storage period are usually a total loss.

RELATION OF SMUDGE TO SHRINKAGE OF SETS IN STORAGE

In order to secure bulbs as nearly comparable as possible except for presence or absence of smudge, healthy and diseased sets averaging about 1 inch in diameter were selected from a general lot of white sets which had been harvested in early August, properly field-cured, and placed in storage on August 22, 1918. Four lots of 25 bulbs each were secured which showed heavy smudge infection but no signs of any other disease. Three lots of 25 each were selected which appeared to be perfectly healthy. All lots were weighed on October 15. Two diseased lots and one healthy lot were kept in the warehouse throughout the experiment under conditions previously described. In order to secure a high relative humidity a special temporary chamber was made in the warehouse and lined with moistened burlap. Thus, a relative humidity of 90 to 95 per cent was maintained at a temperature close to that of the main warehouse. Two diseased and two healthy lots were placed in this chamber for approximately four weeks and then removed to the main warehouse room. The several lots were weighed on December 30, 1918, and on February 18, 1919. The results of the experiment are given in Table VIII. A constant increase in shrinkage of diseased sets over healthy sets was to be noted. Before the end of the experiment sprouting had occurred in most of the lots, and, as was to be expected, was more prevalent in diseased than in healthy lots. Sprouting and the complication of contaminating parasites should be considered; but, since the former is seemingly enhanced by the disease and the latter is not serious in these cases, there is reason to believe that smudge is responsible in large measure for the increase in shrinkage.

TABLE VIII.—*Relation of smudge to shrinkage of onion sets in storage*

Lot No.	Condition of bulbs.	Environment.	Number of bulbs used.	Original weight, Oct. 15, 1918.	Percentage of shrinkage.		Condition at end of experiment.
					Dec. 30, 1918.	Feb. 18, 1919.	
				Gm.			
1	Diseased	Ordinary storage	25	291.8	6.5	18.7	12 sprouting; 1 infected with neck-rot.
18	do	do	25	277.5	7.4	19.0	15 sprouting.
2	Healthy	do	25	319.3	2.5	11.3	8 sprouting; 1 infected with blue mold.
3	Diseased	Exposure to high relative humidity for 4 weeks, followed by ordinary storage.	25	324.0	8.2	23.3	
20	do	do	25	303.0	8.9	28.8	16 sprouting; 3 infected with neck-rot.
4	Healthy	do	25	324.3	2.8	9.1	7 sprouting.
21	do	do	25	284.5	4.1	11.4	5 sprouting.
Average shrinkage of diseased lots					7.7	22.4	
Average shrinkage of healthy lots					3.1	10.6	

SPREAD OF SMUDGE IN STORAGE

It has been claimed that smudge spreads from infected to healthy bulbs in storage (*17*, *29*). It is to be expected that under unusually moist conditions this might occur. However, since considerable moisture is necessary for sporulation and infection, the conditions which prevail in good storage houses are not conducive to rapid spread of the disease. Several experiments have been conducted during the course of this investigation in which healthy bulbs have been marked and mixed in lots of badly diseased sets. A summary of these experiments appears in Table IX.

TABLE IX.—*Spread of smudge in storage*

Experiment No.	Storage conditions.	Length of experiment.	Number of healthy bulbs used.	Condition at end of experiment.
		Days.		
1	Standard onion warehouse.....	154	34	All healthy.
2	.....do.....	103	40	2 bulbs showed slight infection.
3	Cool cellar.....	66	20	All healthy.
4	.....do.....	208	20	Do.
5	Moist chamber at room temperature.	36	20	6 showed slight infection.

It was found that there was little or no spread of the disease under ordinary storage conditions or in a cool cellar. In a saturated atmosphere some infection of healthy bulbs occurred. In practice, then, some spread from diseased to healthy bulbs is to be expected where sets are exposed to rain or very humid atmosphere such as might occur during the curing period. However, with fairly dry sets kept in cool, well-ventilated storage new infections are probably negligible.

CONTROL OF THE DISEASE

The control of this disease is obviously connected closely with the handling of the crop at or immediately following harvest.

In 1915 a spraying experiment was conducted on a plot of white sets at Racine, Wis. The development of the disease in this plot has been described on pages 708–709. Various schedules were used with 4–4–50 and 8–8–50 Bordeaux mixture plus soap, 4–50 copper sulphate, and 1–10, 1–16, and 1–32 lime sulphur. The sprays were applied upon the bulbs and necks of the plants. Contact with the soil probably reduced the disinfecting property of the chemicals, and their adhesiveness was limited by the nature of the scales and leaves of the onion. No beneficial results were secured even where the first application was made before the first signs of the disease appeared and where the spraying was continued at intervals of three to eight days until harvest. The complete failure of

this experiment was sufficient to show that sprays could not be used successfully for the control of smudge.

Dusting of the sets in the crates at harvest time with lime or sulphur has been suggested by Thaxter (*33*). In 1916 and 1918 dusting experiments with lime, sulphur dust, and dry Bordeaux powder were conducted without any positive results. This is to be expected, since, as a rule, the outer scales of the bulbs became infected before harvest and a disinfectant applied externally could hardly prevent further invasion of underlying scales.

The importance of thorough curing and prevention of exposure to humid conditions after harvest has been emphasized by Thaxter (*33*), Clinton (*10, p. 333*), Massee (*17*), and Stevens and True (*30*). The experiments reported on the effect of drying of bulbs at harvest have shown that rapid dehydration of the outer scales at this time checks further invasion by the fungus to a large degree. Observations in the field by the writer during the years 1914 to 1920 indicate that even the best natural curing weather to be expected in the Middle West is not sufficient to do more than partially check the disease on seriously infected fields.

Artificial curing offers a possible measure of control for smudge, and, as already pointed out (*37*), preliminary experiments indicate that neckrot can also be checked by this treatment. Extensive control experiments carried on in the Chicago district in 1918 have shown that thorough drying very soon after harvest is necessary in order to check smudge materially. In the set-growing district a large portion of the crop is grown on contract to be delivered at a central warehouse as soon as it has cured sufficiently. The expense involved in this treatment would almost necessitate that they be dried at a central point, preferably at the place of storage. Therefore, in order to handle the large quantity received, a fairly rapid process of drying would be essential.

Further experimental work is necessary before artificial drying can be recommended as a general practice, and the results of control experiments are reserved for later publication. In the meantime, the most applicable remedial measures consist in prompt harvest and the best use of natural climatic conditions in curing the white onion set crop, including all possible protection from moist weather. This should be followed by storage in a well-ventilated warehouse held as nearly as possible at 33° to 36° F.

SUMMARY

(1) Smudge is one of the most common diseases of white onion sets in Wisconsin and Illinois.

(2) It occurs also on shallot (*Allium ascalonicum*) and leek (*A. porrum*).

(3) The disease was first described by Berkeley in England in 1851 and is now widely distributed in Europe and America.

(4) Smudge is confined to the scales and neck of the bulb, where it causes dark green to black spots. On fleshy scales it appears as sunken yellowish spots which enlarge slowly, coincident with gradual shrinkage of the scale. On colored varieties the disease is confined to unpigmented portions of the outer scales of the neck of the bulb.

(5) Spots on the outer scales of bulbs due to *Macrosporium porri*, *M. parasiticum*, *Phoma alliicola*, and *Urocystis cepulae* may be confused with smudge.

(6) Smudge becomes detrimental to the onion crop as a cause of (1) the reduction of market value of white varieties, (2) shrinkage in storage, and (3) premature sprouting of sets in storage.

(7) A detailed description of the morphology of the causal organism, *Colletotrichum circinans* (Berk.) Voglino, is given. The ascigerous form, *Cleistothecopsis circinans*, has been described by Stevens and True, but complete proof of its connection with *Colletotrichum circinans* is lacking.

(8) Inasmuch as the causal organism produces a subcuticular stroma and a well-defined acervulus, the species is classified in the Melanconiales as *Colletotrichum circinans* (Berk.) Voglino. A comparative study of the latter with *C. fructus* (S. and H.) Sacc. was made.

(9) The characteristic growth of the organism on culture media is described.

(10) Growth on potato agar takes place between 2° and 32° C., while the optimum is about 26°.

(11) Spore germination is stimulated in soil decoction, onion decoction, and sterilized soil extract, as compared with that in distilled water, while it is reduced in unsterilized soil extract and entirely inhibited in onion leaf or scale extract.

(12) Spore germination occurs within the range of 4° and 32° C., while the optimum temperature is from 20° to 26°.

(13) Conidia are very sensitive to desiccation except when in spore masses, in which condition a small percentage retain vitality for four months or more. Stromata are very resistant to desiccation, retaining vitality for two years or more.

(14) Conidia are sensitive to freezing temperatures, but dried spore masses may withstand this environment for a month or more. Stromata are capable of withstanding several months of freezing weather.

(15) The fungus is pathogenic upon the scales of mature bulbs, but does not attack actively growing parts of the plant with the exception of young seedlings, upon which it may cause "damping off" under certain greenhouse conditions.

(16) Spores germinate and appressoria form within 10 to 12 hours. The infection tube is pushed from the side of the appressorium adjacent to the host cuticle directly through the latter. The mycelium then develops for a time between the cuticle and the subcuticular wall, raising

the former and eventually causing a softening of the latter. In bulbs inoculated in moist chambers the fungus progresses fairly rapidly, causing softening and lamination of the walls and the gradual collapse of the cell. The stroma involves the subcuticular wall at first and later the underlying cells, but the cuticle remains unbroken until the acervulus is formed. The process of invasion under storage conditions is essentially the same but much slower.

(17) The fungus overwinters as stromata in infected scales.

(18) Infection occurs at or above 10° C., but progress is very slow below 20°; the optimum is about 26°.

(19) Conidia are produced abundantly under moist conditions and at temperatures between 20° and 30° C. They are disseminated chiefly by meteoric water, especially spattering rain.

(20) The disease develops most rapidly in the field when the mean soil temperature range lies between 20° and 30° C. and is accompanied by abundant rainfall. Extremely hot, dry weather in July checks progress. Presence of moisture favors the progress of the disease during the curing period, whereas artificial drying of sets immediately following harvest checks it.

(21) Smudge tends to promote premature sprouting and increases shrinkage of sets in storage. The disease may spread from bulb to bulb in the crate under very moist conditions, but in proper storage this factor is negligible.

(22) The important measures of control are protection of the harvested crop from rain, rapid and thorough curing, and provision of well-ventilated storage at about 33° to 36° F.

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PLATE 80

Onion smudge:

Onion sets (White Portugal variety) naturally infected with *Colletotrichum circinans*. Collected on August 27, 1919, several weeks after harvest, at Morton Grove, Ill. Photographed September 23, 1919. Note in the three lower bulbs the small sunken spots in the fleshy scales which mark the early stages of invasion of the living tissue. Natural size.



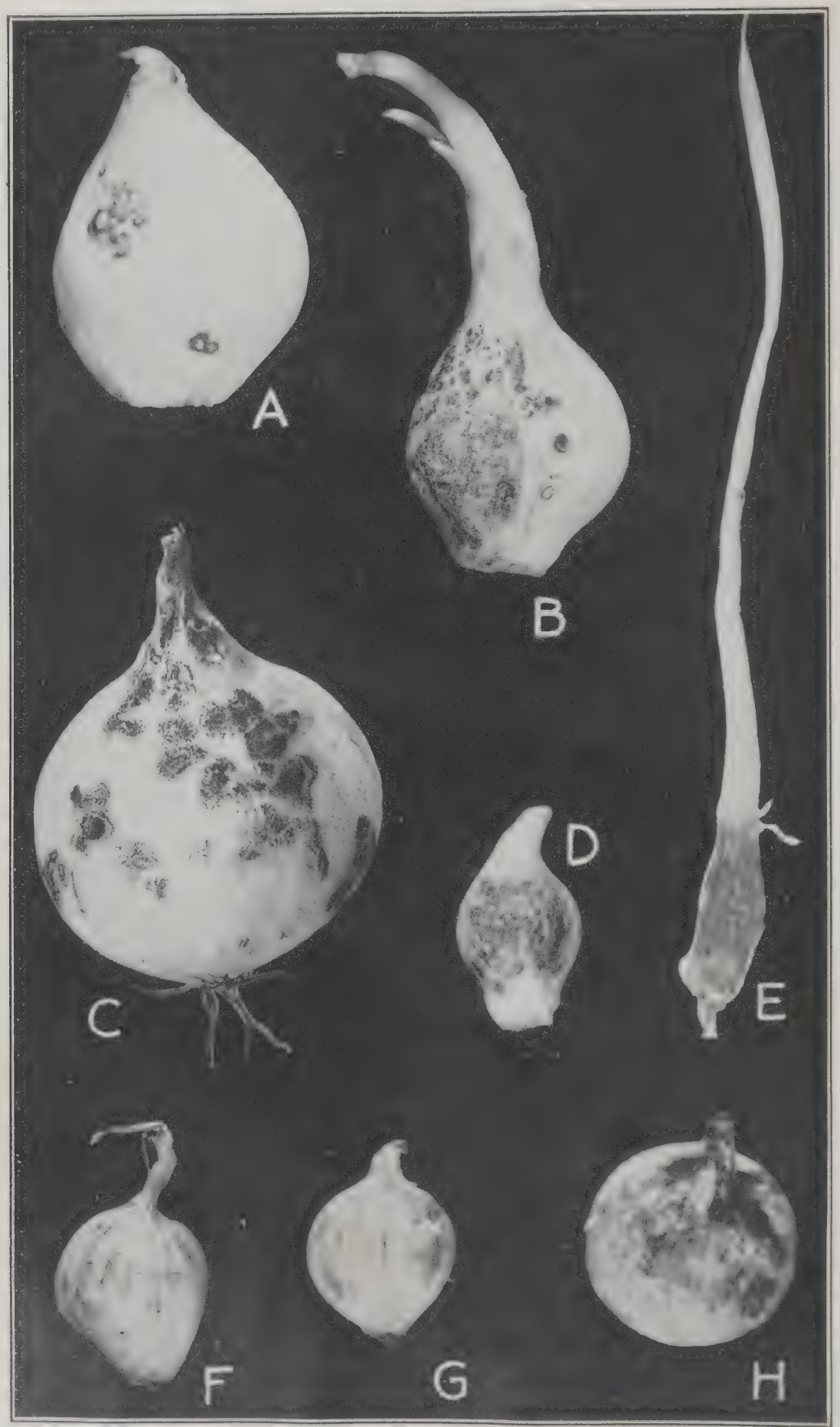

A
B
C
D
E
F
G
H

PLATE 81

Onion smudge:

A, B, E, D.—Advanced stages of smudge after several months in storage. Note the shrinkage of fleshy scales and the tendency to sprout.

C.—Bulb inoculated in a moist chamber with a suspension of *Colletotrichum circinans* conidia.

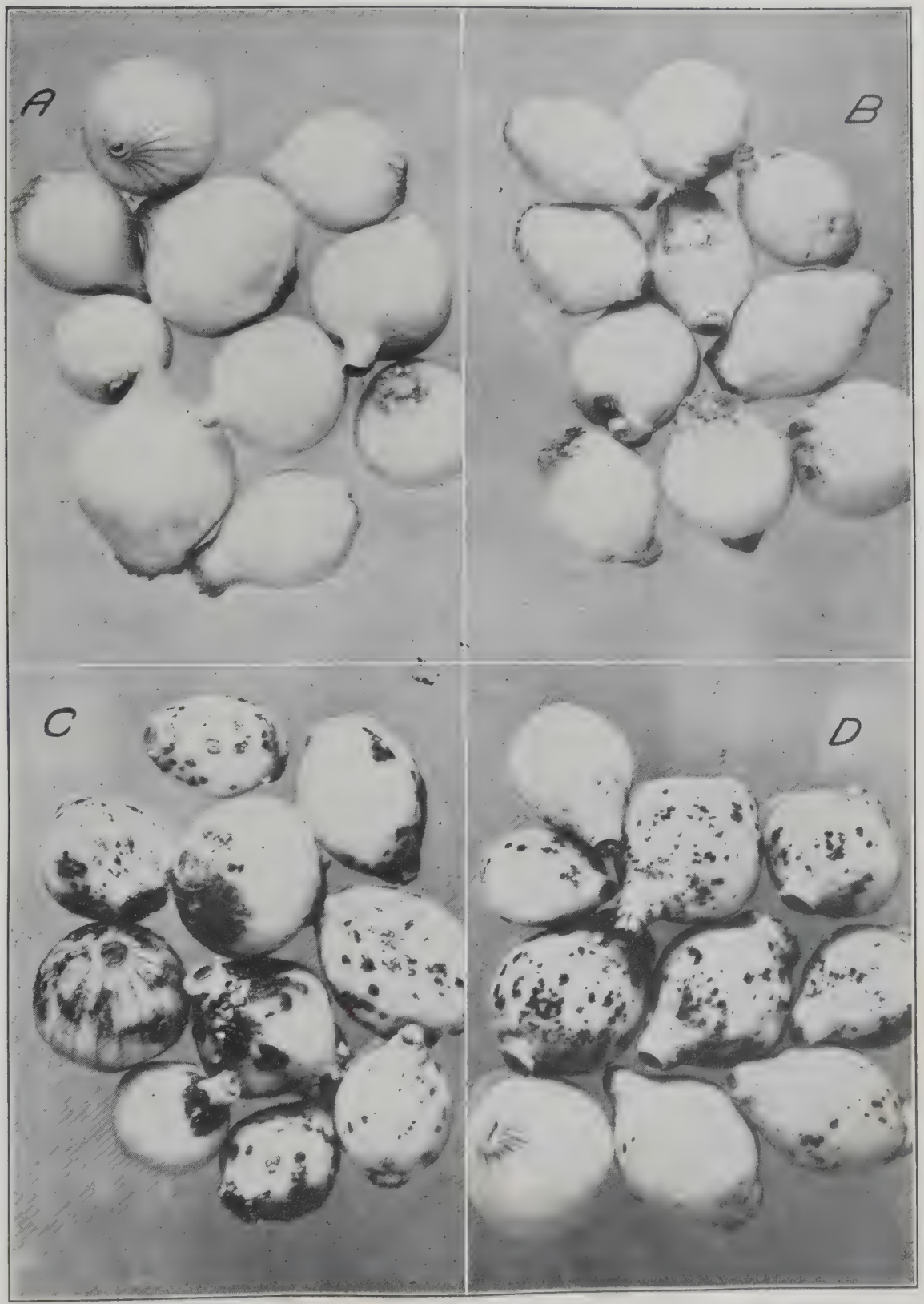
F, G.—*Macrosporium* sp. on outer scale of white onion sets.

H.—*M. porri* and *Phoma alliicola* on outer scale of white onion set. Natural size.

PLATE 82

Relation of soil temperature to the development of smudge:

Onions kept in infected soil held at different temperature for nine days.
A.—5° C.
B.—15° C.
C.—23° C.
D.—32° C.
Slightly reduced.


A
B
C
D

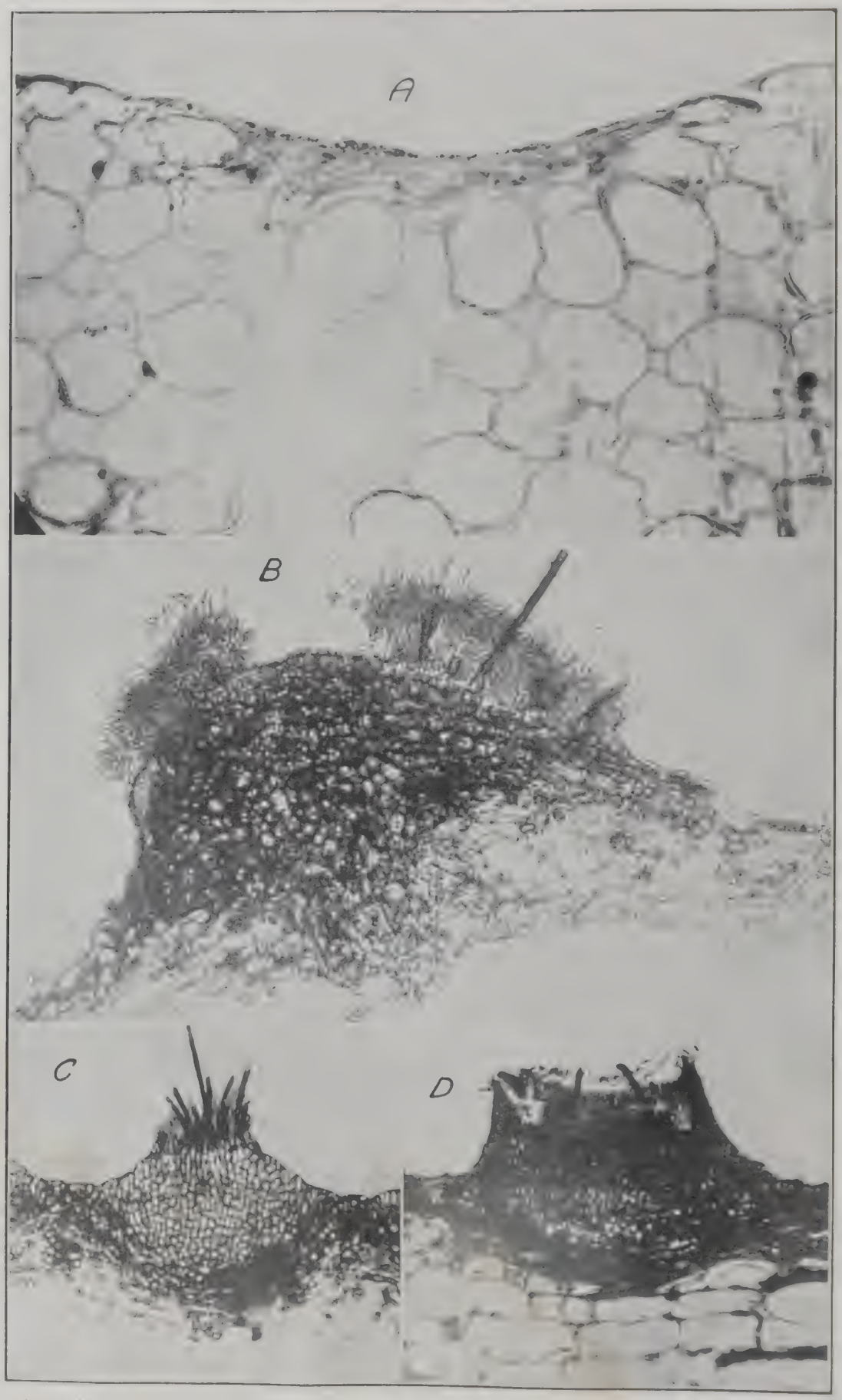

A
B
C
D

PLATE 83

Colletotrichum circinans and *C. fructus:*

A.—Photomicrograph of cross section of naturally infected onion scale. Note that the fungus is confined largely between the cuticle and the subcuticular wall. The epidermal cells and two layers of the parenchyma cells have collapsed, while the uninvaded cells beneath the lesion are slightly enlarged and distended.

B.—Photomicrograph of cross section of an infected onion scale held for several months in poorly ventilated storage. Note that the stroma is excessively developed and that the cuticle is still intact except where ruptured by the acervuli.

C, D.—Photomicrographs of cross sections of *C. circinans* (C) and *C. fructus* (D) on apple fruit. Note similarity between the two forms and the subcuticular origin of the stromata in each case.

PLATE 84

Colletotrichum fructus and *C. circinans:*

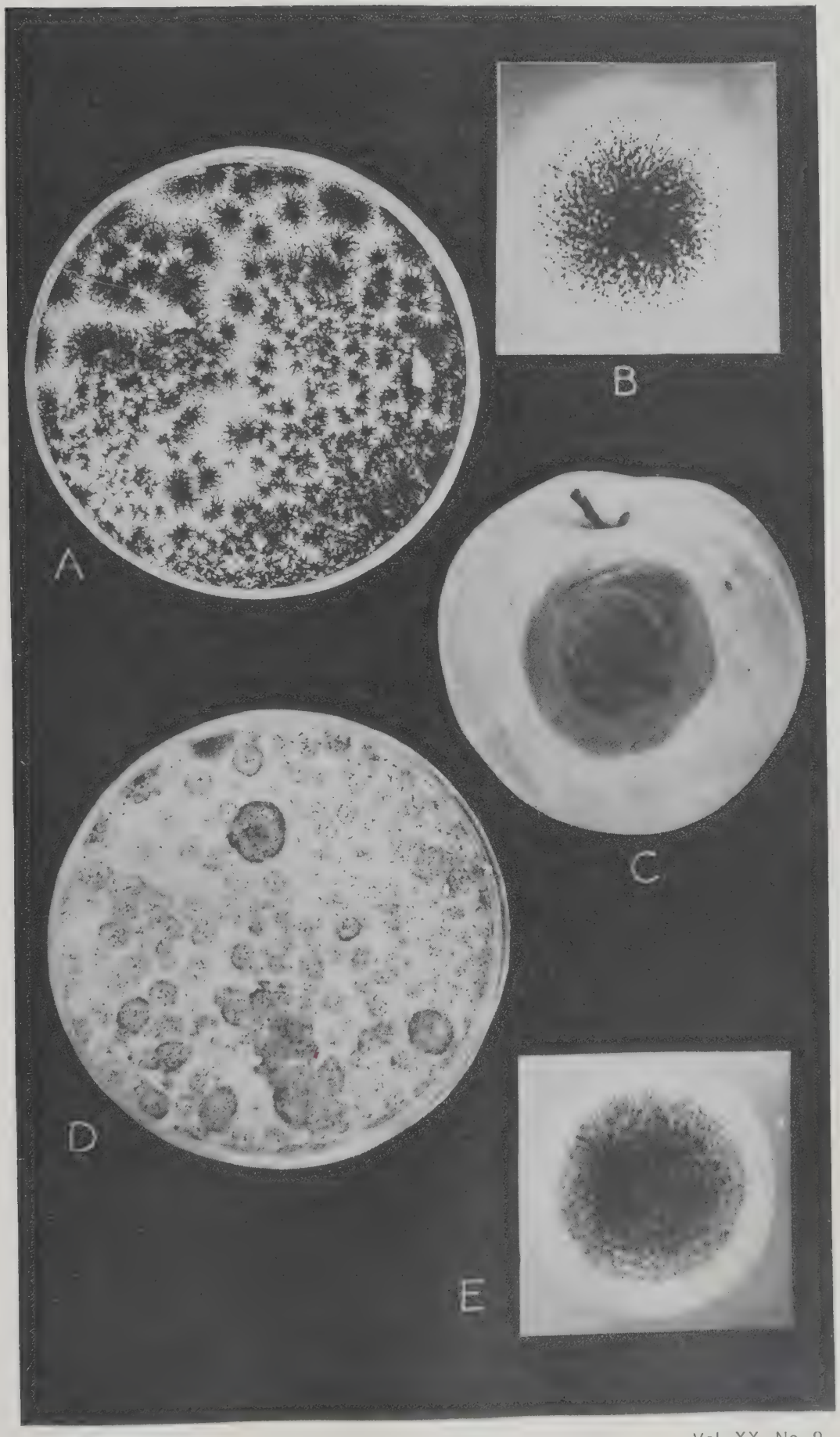
A.—Dilution plate from spores of *Colletotrichum fructus*. Photographed on sixth day. Note stellate character of colonies as compared with *C. circinans* in D. × 4/5.

B.—Individual colony of *C. fructus* on potato agar. Photographed on the fourth day. Compare with *C. circinans* in E. × 1¾.

C.—Apple of Fameuse variety inoculated with mycelium from pure culture of *C. circinans*. Photographed two months after inoculation.

D.—Dilution plate from spores of *C. circinans*. Photographed on sixth day. Compare with *C. fructus* in A. × 4/5.

E.—Individual colony of *C. circinans* on potato agar. Photographed on fourth day. Compare with *C. fructus* in B. × 1¾.


A
B
C
D
E

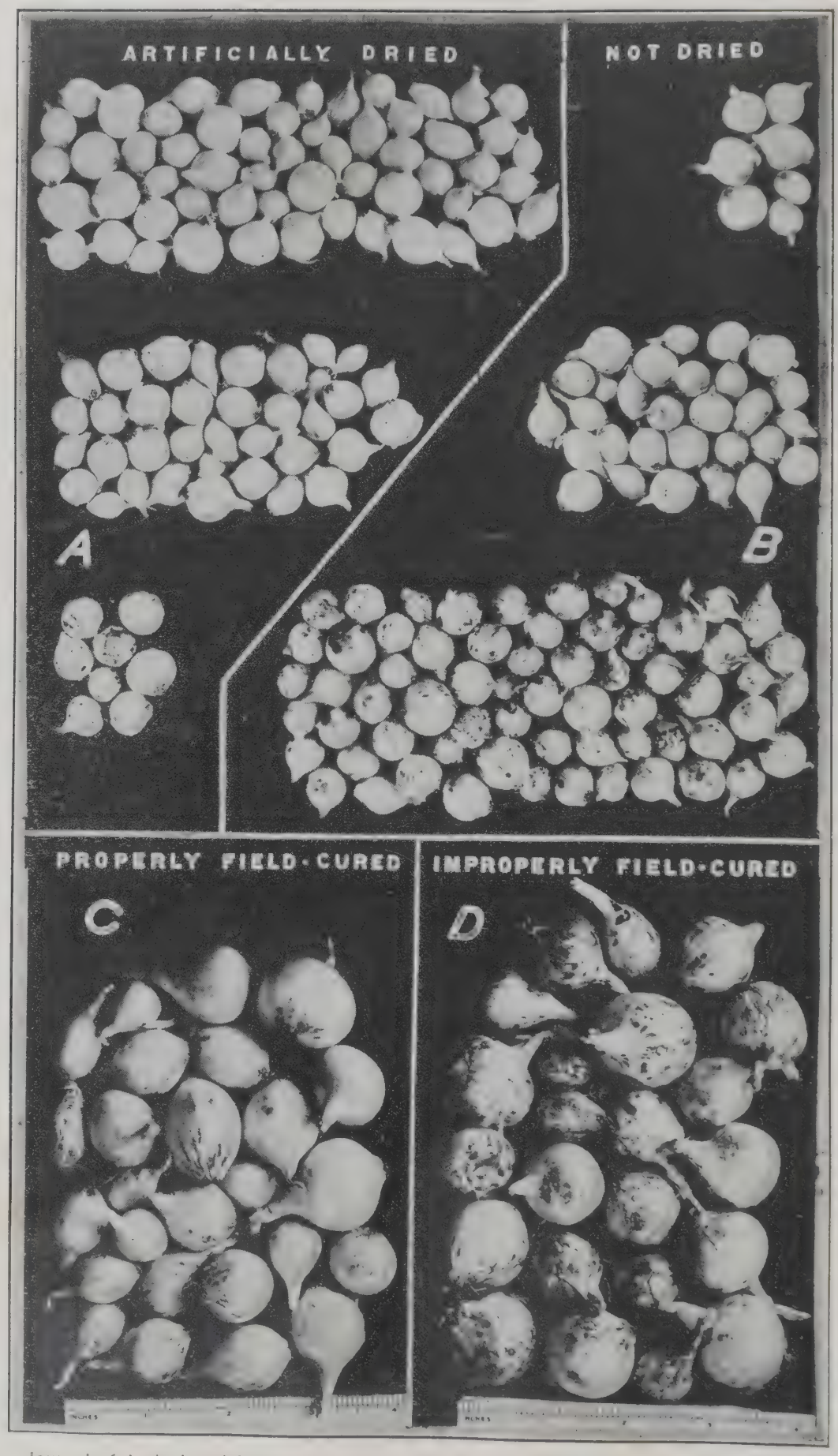

ARTIFICIALLY DRIED
NOT DRIED
A
B
PROPERLY FIELD-CURED
IMPROPERLY FIELD-CURED
C
D

PLATE 85

Relation of curing conditions to the development of smudge:

A, B.—Comparison of onion sets artificially dried immediately after harvest with those not dried. Photograph made at the end of the storage period after the two lots had each been divided into three classes—namely, those free from disease, those slightly diseased, and those badly diseased. (See experiment 1, p. 710–711.)

C, D.—Comparison of white onion sets cured in shallow crates in the field under the best of natural conditions with part of the same lot after exposure to moist conditions for one week. (See experiment 2, p. 711–712.)

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VARIATIONS IN COLLETOTRICHUM GLOEOSPORIOIDES[1]

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The diseases of citrus trees and fruit known as wither-tip, leafspot, anthracnose, and tearstain are all caused by the same fungus, *Colletotrichum gloeosporioides* (Penz.). These diseases have been found in Florida, (*4; 5; 9, p. 88*),[3] California (*3*), West Indies, South America, Australia, and Malta; and in practically all citrus-growing regions rather serious outbreaks of some or all of these diseases have occurred from time to time.

The smaller twigs of citrus trees are very frequently and severely attacked by the fungus. It is quite common to see many of the small twigs killed back 4 or 5 inches. These infected twigs soon turn to a light brown color and sooner or later become dotted over with numerous small black acervuli. After the rainy season begins, the spores, which are imbedded in a gelatinous matrix, exude from the acervuli and are washed down over the fruit and leaves, causing leafspot, tearstain, and anthracnose of the fruit.

The spores must have an abundance of moisture in order to germinate. Since the rainy season in California occurs during the winter and early spring months, it is at this period that these diseases are most prevalent. In Florida these diseases cause much damage to the citrus industry, whereas in California they are considered of minor importance. This difference in the amount of injury in the two States named is due, I believe, to the difference in the amount of rainfall. During the dry summer in California there is little evidence that *Colletotrichum gloeosporioides* is active. In Florida this fungus causes bloom drop and a considerable amount of leaf spotting during the spring and summer months, as well as anthracnose and tearstaining of the ripe fruit. Many growers and agricultural workers believe that the fungus injury is secondary. It has been stated repeatedly that the weak or injured tree is more susceptible to an attack of *C. gloeosporioides* than the healthy tree.

DESCRIPTION AND HISTORY OF THE FUNGUS

The fungus, *Colletotrichum gloeosporioides* (Penz.) was first described by Penzig in 1882 as *Vermicularia gloeosporioides*. In 1887 he placed

[1] Paper No. 66, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Resigned June 1, 1918.

[3] Reference is made by number (italic) to "Literature cited," p. 735-736.

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it in the genus Colletotrichum. It was first collected in America in 1886 by Dr. Martin from Green Cove Springs, Fla., and was first reported by L. M. Underwood (*8*) in 1891. However, the disease was not found in California until some years later. It was reported by Essig (*4*) in 1909 from the Limoneira Ranch at Santa Paula, where it was causing considerable damage to lemon trees.

In 1904, Prof. P. H. Rolfs (*5*) gave a very good description of the fungus as it occurred on various citrus trees and fruits in Florida. He says (*5, p. 20*) that the—

> diseases . . . manifest themselves as wither-tip on orange, pomelo, and lemon twigs; as leaf-spot on the leaves of the various citrous species; as anthracnose on lime blossoms, recently set limes, lime twigs, and lemon twigs; as lemon-spot on ripe lemons and as canker of limes.

The following description is given by Prof. P. H. Rolfs:

> Acervuli located on the surface of the leaf, twig or fruit; 90–270 μ in diameter, erumpent, superficial. Shape various, not uniform, occurring on either surface of citrus leaves; disposed irregularly or in more or less concentric lines; pale to dark colored. On tender lime twigs, tender lemon twigs, lemon fruits and lime fruits, pale colored, dull red in masses, confluent. Epidermis breaks irregularly. Setae fuliginous, ranging in length from 60–160 μ, frequently once or twice septate, disposed at margin of acervuli. Frequently absent, and on tender lime twigs, tender lemon twigs, lemon fruits and lime fruits usually absent.
>
> Conidia broadly oval or oblong, 10–16 μ by 5–7 μ, hyaline; size variable in same acervulus, usually with one or two oil drops. Developing from a well-defined stroma; basidia, 3–18 μ. In moist chambers the conidia stream from the break in the epidermis. Intrabasidial setae, variable 8–30μ by 3–6μ, cylindrical or sometimes enlarged at distal end; hyaline.

In 1912 Clausen (*1*) described the fungus causing wither-tip of the lime, *Citrus medica*, as *Gloeosporium limetticolum*. He believes that Rolfs had confused two forms and described them as one. Clausen uses the absence of setae as a distinguishing character from *Colletotrichum gloeosporioides*. It is the opinion of Stoneman (*7*), Edgerton (*2*), and Shear and Wood (*6*) that the setae are variable as to presence or absence and that they are not reliable morphological characters to use in separating genera. I have found them in some of my cultures of *Colletotrichum gloeosporioides*, while in other cultures they were absent. Another character he uses is the lack of a coarsely granular plasma filling the spores. I have found several strains of this fungus which are considered to be *Colletotrichum gloeosporioides*, whose spores are not filled with a coarse granular plasma but appear at first to be homogeneous. Clausen also uses growth characteristics as a means to identify the two strains. Some of my strains had the same growth characteristics as the strain which was obtained from Clausen—that is, a white mycelium and abundant spore production.

Shear and Wood (*6*) in their bulletin on the genus Glomerella, have brought together strains from various hosts and included them in one

species, *Glomerella cingulata*. To my knowledge, the perfect stage of Clausen's fungus has not been found. Several of my strains produced the perfect stage when first isolated, and the spores and asci were the same as described for *G. cingulata*. It is, therefore, the opinion of the writer, which will be presented in the following pages, that *Colletotrichum gloeosporioides* as found in California is a polymorphic species, composed of many strains.

STRAINS IN COLLETOTRICHUM GLOEOSPORIOIDES

In the fall of 1916 when the writer began work at the Citrus Experiment Station, the wish was expressed that he should study *Colletotrichum gloeosporioides*. The different members of the Division of Plant Pathology had isolated several cultures of this fungus from different citrus hosts. Some of these differed from each other in their cultural characteristics. It was suggested that these forms might have different regional distribution, or that their differences might be due to the host. Other isolations were made from the various citrus hosts; and these, together with the cultures obtained from the different members of the Division of Plant Pathology, were given laboratory numbers and were always spoken of as strains. In all, 46 cultures were used in the study. Forty-two of these represented all the important citrus districts of southern California, and there was one each from Texas, Florida, Alabama and one kindly furnished by Dr. C. L. Shear.

CULTURAL CHARACTERISTICS

The various strains were grown on five different media—corn meal agar, green bean plugs, potato agar, lactose-beef agar and oatmeal agar. Each strain was grown on these five different media for a period of 18 months. Transfers were made about every 5 weeks, and a record was kept of the variations in growth occurring in each strain on the various media. While most of the strains exhibited different cultural characteristics on the various media, there were a few whose macroscopic characteristics of the mycelium were much the same on all the media. Not only did each strain vary in its growth characters on the different media but some of the strains differed characteristically from each other. Therefore, the variations exhibited by the various strains in their cultural characteristics made it possible to classify them into the following five groups.

Group I: Mycelium white; spores abundant, salmon-colored in mass.

Group II: Mycelium grey to greenish black on the various media, very little aerial growth on oat agar; spores abundant, salmon-colored or yellowish in mass.

Group III: Mycelium gray to black on various media; no spore masses on oat agar.

Group IV: Mycelium gray to black; spore production so abundant on all media that the surface of the medium is nearly covered by a bacteria-like mass of spores.

Group V: Mycelium gray to black, rather fluffy; no pink spore masses on any medium; spore production scant and on some media no spores produced.

Since the cultural characteristics of some strains changed, it became necessary to reclassify the different strains on the following dates: January 27, 1917; April 16, 1917; September 13, 1917; and February 28, 1918. Very few of the strains remained in the group in which they were placed at the first classification. Under artificial cultivation the characteristics of the various strains changed; therefore, they were placed in different groups (see Table I). There were only three strains whose characteristics remained constant in group I. In group II there was only one strain which remained constant. It will be noticed that in group IV cultures 296 and 299 remained constant until September 13, 1917. At the next date of classification these two strains were placed in group II. No strains were placed in group V until September 13, 1917. This may be due to the fact that under artificial conditions these strains lost their power to produce spores.

TABLE I.—*Classification of strains of Colletotrichum gloeosporioides into groups*

Group No.	Jan. 22, 1917.			Apr. 16, 1917.			Sept. 13, 1917.			Feb. 28, 1918.		
I	[a] 295			[a] 295			[a] 295	[a] 429	934	[a] 295		
	[a] 298			[a] 298			[a] 298	496	955	[a] 298		
	323			323			323	502		[a] 429		
	[a] 429			[a] 429			326	901		496		
										561C		
II	326	475	[a] 990	326	560	[a] 990	325	[a] 990		296	527	926
	459	483		459	561		475			299	536	[a] 990
	496	651		475	651		483			323	536A	
	502	901		483	912		510			325	536B	
	507	912		502	926		536			326	561B	
	510	926		507	934		612			502	620	
	943	940		536	943		615			510	901	
III	297	934		297	496	955	507	912		406	536C	912
	325	955		325	510		560	943		475	560	943
	406			406	910		561			507	561	955
	467			467	940		651			527A	561A	
											527C	612
IV	296			296			296					
	299			299			299					
V							297			297	934	
							467			483	940	
							495			495		
							527			527B		
							940			651		

[a] Culture remained in its original class throughout the work.

VARIATIONS IN SPORE LENGTH

Since such great differences were found in cultural characteristics between the strains, the question arose whether differences could be found in the spore length of the various strains. One hundred spores were measured from each strain. The measurements were made in the fol-

lowing manner: A dilute suspension of the spores taken from green bean plugs was made in sterilized tap water, and a drop of the suspension was placed on a microscope slide and covered with a cover glass. It was necessary to make the measurements quickly, because the spores did not remain quiet for any length of time. The image of the spore was thrown on drawing paper by means of the camera lucida, and the length and width were quickly marked with a pencil. The microscope was so adjusted that 1 micron on the micrometer scale in the eyepiece was equal to 1 millimeter on the paper. Therefore, after the length and width were indicated on the paper the spore size could be quickly ascertained by means of a millimeter rule.

TABLE II.—*Variation in spore length in the different strains of Colletotrichum gloeosporioides*

Strain No.	Number of spores measuring (in microns)—																			
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
296	1	1	3	9	19	28	18	14	6											
901									1	1	7	10	12	21	19	16	10	1	1	1
459						4	5	26	33	27	4	1								
429				1	1	2	10	29	31	18	5	2	0	1						
406				1	0	2	4	4	13	21	27	11	12	1	2	2				
326							3	4	15	25	28	15	8	0	1					
955				1	5	23	17	29	21	2	0	1								
295				1	4	14	16	20	28	8	7	2								
990	1	0	0	0	1	1	2	8	35	25	19	5	3							
297						4	4	25	27	23	14	1	2							
651					1	1	0	1	8	16	33	21	12	4	3					
299						15	16	20	32	13	3	0	1							
323					6	24	33	23	11	1	2									
510						3	4	15	41	19	9	7	1	1						
507						7	22	27	37	6	1									
502						13	32	38	13	2	1	0	1							
943						4	6	17	26	18	16	11	2							
940							6	25	37	21	8	2	1							
934				2	0	5	10	18	32	16	16	0	1							
527			5	16	21	22	11	8	5	3	5	4								
560						11	16	23	28	13	2	3	3	0	1					
536						1	2	9	21	30	20	12	4	0	1					
561						2	3	12	36	20	20	4	1	2						
524							2	5	22	39	20	10	2							
514						2	1	9	20	33	19	11	5							
513							1	8	24	43	13	8	3							
517						1	1	8	19	44	18	8	1							
515							1	2	8	21	28	22	13	3	2					
947							2	3	19	35	27	12	0	0	2					
467						1	2	6	22	21	25	13	9	1						
512							4	4	20	36	27	5	2	1	0	1				
475						1	2	7	22	26	26	12	2	1	1					
926							3	16	31	27	17	5	1							
483						2	2	8	26	28	21	10	1	1	0	1				
325			1	0	0	7	11	39	30	9	2	0	0	1						
298				1	0	5	9	24	31	21	6	3								

It was soon determined that each strain had a certain range of variability in its spore length and width (see Table II). While there were

individual variations exhibited, yet it was soon determined that many of the strains had the same mode for their spore lengths. Therefore, the cultures were classified in regard to the modal length of the spores (see Table III). The strains varied in their modal spore length from 12 to 20 μ. Most of the strains have their mode at 15 μ.

TABLE III.—*Mode of the spore length of different cultures of Colletotrichum gloeosporioides*

Spore No. measuring (in microns)—								
12	13	14	15	16	17	18	19	20
296	323	325	295	475	326	.....	.....	901
527	.....	502	297	483	406	.....	.....	.....
.....	.....	955	298	512	467	.....	.....	.....
.....	.....	.....	299	513	515	.....	.....	.....
.....	.....	.....	429	514	651	.....	.....	.....
.....	.....	.....	459	517	912	.....	.....	.....
.....	.....	.....	507	524	.....	.....	.....	.....
.....	.....	.....	510	536	.....	.....	.....	.....
.....	.....	.....	560	947	.....	.....	.....	.....
.....	.....	.....	561	.....	.....	.....	.....	.....
.....	.....	.....	926	.....	.....	.....	.....	.....
.....	.....	.....	934	.....	.....	.....	.....	.....
.....	.....	.....	940	.....	.....	.....	.....	.....
.....	.....	.....	943	.....	.....	.....	.....	.....
.....	.....	.....	990	.....	.....	.....	.....	.....

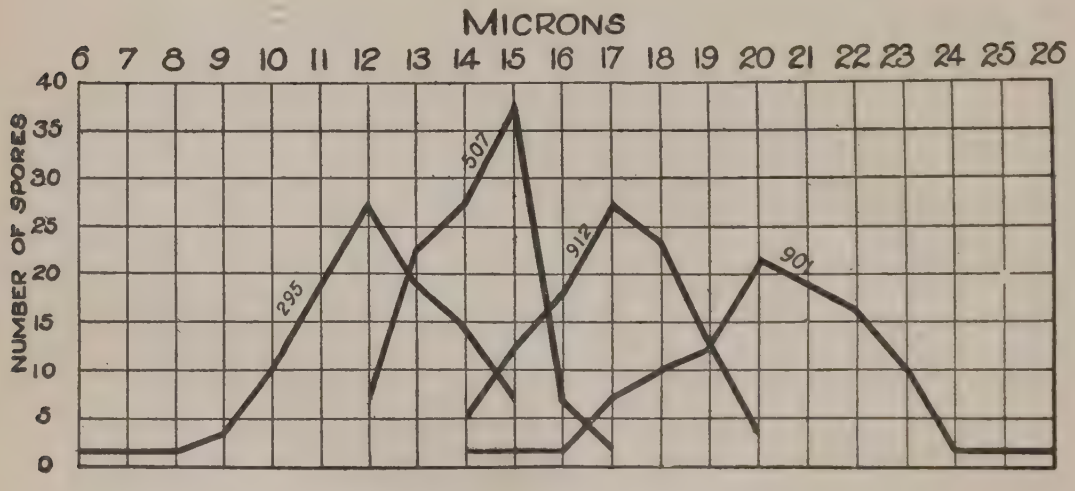


FIG. 1.—Variability of strains of *Colletotrichum gloeosporioides* in spore length.

It was soon observed that this classification could not be correlated with the classification of the strains based on their cultural characteristics. It was hoped that it would be possible to find morphological differences correlated with the cultural characters, but this was not the case.

In order to show the variability within the strain and the differences between the strains, graphs were made representing the variability in four strains (fig. 1). Strain 296 has its modal spore length at 12 μ, 507

has its mode at 15 μ, 912 has its mode at 17 μ, and strain 901, which has the largest spores of all the strains, has its mode at 20 μ.

There was also a certain range of variability in spore width. The variability was not as great as in length. The widths ranged from 3 to 8.5 μ; in most of the strains the mode was about 4 or 5 μ. In strain 901 the variability was from 5 to 8.5 μ with the mode at 6.5 μ.

In Table IV are given the calculated mean, standard deviation, and probable error of each, for the spore length and width of eight different strains. The measurements were made from spores taken from the green bean plug medium.

TABLE IV.—*Table of calculated spore measurements for certain strains of Colletotrichum gloeosporioides*

Strain No.	Mean length of spore in microns.	σ	Mean width of spore in microns.	σ
295	11. 54±0. 065	0. 97±0. 046	5. 52±0. 057	0. 85±0. 041
296	12. 01± . 115	1. 71± . 082	4. 2 ± . 065	. 97± . 046
298	14. 79± . 094	1. 40± . 067	4. 68± . 014	. 21± . 010
429	14. 73± . 095	1. 42± . 068	3. 26± . 077	1. 15± . 055
507	14. 16± . 079	1. 17± . 056	4. 91± . 048	. 71± . 034
651	17. 23± . 110	1. 64± . 078	4. 52± . 035	. 52± . 025
901	20. 34± . 137	2. 04± . 097	6. 45± . 132	1. 96± . 093
912	16. 99± . 097	1. 44± . 069	4. 7 ± . 110	1. 63± . 078

This table shows that strains grown on the same medium under like conditions vary greatly in respect to their spore sizes. We can, therefore, safely conclude that there exist individual differences in the various strains in regard to certain morphological characters.

VARIATIONS IN THE DIFFERENT STRAINS INDUCED BY THE MEDIUM

The difference in growth characteristics occurring in the same strain when transferred to the various media was very noticeable. The various strains were grown on the five different media for a period of one year. Transfers were then made from cultures growing on the various media to different plates poured with the same medium. The plates were kept at room temperature, and their growth characteristics were noted. It was soon observed that some strains had been more affected than others by their previous environment. While some of the variations were slight, still it was impossible to account for this variation other than as the effect of the medium.

On October 25, 1917, 20 cc. of potato agar were poured in sterilized Petri dishes and allowed to harden. Transfers were then made from the various strains as follows:

STRAIN 429

Plates 1 to 4 were transfers from mycelium on corn meal agar.
Plates 5 to 8 were transfers from spores on corn meal agar.
Plates 9 to 12 were transfers from mycelium on green bean plugs.
Plates 13 to 16 were transfers from mycelium on glucose-potato agar.
Plates 17 to 20 were transfers from mycelium on lactose-beef agar.
Plates 21 to 24 were transfers from mycelium on oatmeal agar (spores).
Plates 25 to 30 were transfers from mycelium on oatmeal agar (mycelium).
On November 22 the final notes taken on the foregoing cultures were as follows:
Plates 1 to 4. White, woolly fungal growth covering the medium. Plate No. 4 was distinctly zoned; spores in center of culture.
Plates 5 to 8. White, scanty fungal growth, which gave the culture a granular appearance; spores in center of culture.
Plates 9 to 12. White, cottony growth, not zoned, but in two plates there was considerable dark mycelial growth; spores in center of culture.
Plates 13 to 16. Very scanty white mycelial growth; few spores.
Plates 17 to 20. White, cottony growth; no spores.
Plates 21 to 24. A membrane-like growth over the entire surface. Very little aerial growth; few spores.
Plates 25 to 30. White, scanty growth of a granular appearance; zoned.

STRAIN 561

Cultures made on glucose potato agar, December 18, 1917.
Plates 1 to 5 were transfers from corn meal agar.
Plates 6 to 10 were transfers from glucose-potato agar.
Plates 11 to 15 were transfers from oatmeal agar.
The final notes were taken on December 28, 1917.
Plates 1 to 5. There is a gray, woolly aerial mycelium; growth in medium is dark In plate 1 there is a white sector; no aerial growth but abundant spore production.
Plates 6 to 10. The growth is white, apprest, wet-looking; no spores.
Plates 11 to 15. No aerial mycelium, zoned, growth in medium white; good spore production on surface.

STRAIN 560

Cultures were made on Petri dishes, poured with corn meal agar December 5, 1917.
Plates 1 to 3 transferred from corn meal agar tubes.
Plates 4 to 6 transferred from green bean plug.
Plates 7 to 9 transferred from glucose-potato agar.
Plates 10 to 12 transferred from lactose-beef agar.
Plates 13 to 15 transferred from oatmeal agar.
On December 17 the final notes taken on the foregoing cultures were as follows:
Plates 1 to 3. White growth in medium; good spore production.
Plates 7 to 9. White growth in medium; no aerial growth; no spores.
Plates 10 to 12. White, woolly aerial growth; no spores.
Plates 13 to 15. Growth in medium, dark; very scant aerial growth; no spores.

STRAIN 990

On October 16, 1917, corn meal agar plates were inoculated with strain 990, the transfers being made from the various media.
Plates 1 to 4 transferred from corn meal agar tube.
Plates 5 to 8 transferred from green bean plug.
Plates 9 to 12 transferred from glucose-potato agar tube.

Plates 13 to 16 transferred from lactose-beef agar tube.
Plates 17 to 20 transferred from lactose-beef agar tube.
Plates 21 to 24 transferred from oatmeal agar (mycelium).
Plates 25 to 28 transferred from oatmeal agar (spores).
The final notes were taken October 29, 1917.
Plates 1 to 4. Gray, short mycelial growth.
Plates 5 to 8. Gray to black aerial mycelium, but in some spots there were no aerial hyphae, growth confined to the medium; good spore production. The peculiar spots were more or less in sector-like areas. Plate 6 showed definite sectors of black and gray aerial mycelium, and in some sections the growth was confined in the medium.
Plates 9 to 12. Almost all the plates had a good growth of gray aerial mycelium, while in others there appeared sectors where the mycelium was confined in the medium.
Plates 13 to 16. No aerial mycelium, but the growth was confined in the medium, was light-colored, and was producing many spores.
Plates 17 to 20. The aerial growth is gray, woolly; some spores produced.
Plates 21 to 24. Gray felt-like growth covering the medium; no spore production.
Plates 25 to 28. These plates differed from plates 21, 22, 23, and 24 in that some of the plates were zoned and produced more spores.

It is clear that there exist variations in a single strain which can not be accounted for on any other ground than the effect of environment. If, therefore, the differences in environment have caused these variations in one year, there may be a possibility of certain environments causing still greater variations which would be more or less permanent.

EFFECT OF THE MEDIUM ON SPORE SIZE

Spores were also measured from the different media to ascertain whether the spore size had been affected. One hundred spores were measured from five different media, and the mean length, mean breadth, standard deviation, and probable error of the mean were calculated for five strains (see Table V). It will be seen that the various media did affect the spore size, but all strains were not affected alike by the same medium. While it has been definitely shown that there exist different strains in *Colletotrichum gloeosporioides*, it also has been shown that these strains are affected in growth characteristics and morphological characters by the medium.

MUTATIONS

The variations which have been described in this paper occurring in the various strains of *Colletotrichum gloeosporioides* have been shown to be due to environmental factors. Not all the variations, however, which occurred during the progress of the work are thought to be due to the environment. These variations which were thought to be induced by some factor or factors other than the environment are in this paper called mutations. These mutations have kept their peculiar characteristics although grown under the same conditions as the cultures from which they arose.

When the various strains were isolated in the fall of 1916, they were grown in plate cultures to study their growth characteristics. The

cultures in which the mutations occurred had greenish gray, fluffy, aerial growth. None of the cultures showed any variation from the description given in the table. This seems to indicate that the cultures were all pure.

TABLE V.—*Differences in spore size of Colletotrichum gloeosporioides induced by various media*

Kind of medium.	Mean spore length in microns.	σ	Mean spore width in microns.	σ
STRAIN 295				
Corn meal agar	11.54 ±0.065	0.97±0.046	5.52±0.057	0.85 ±0.041
Green bean plug	14.13 ± .114	1.69± .081	4.41± .11	1.63 ± .078
Potato agar	13.6 ± .129	1.92± .092	4.9 ± .055	.835± .040
Lactose agar	15.74 ± .319	4.74± .226	4.65± .044	.65 ± .031
Oat agar	13.24 ± .071	1.06± .051	5.34± .018	.27 ± .013
STRAIN 296				
Corn meal agar	9.14 ±0.111	1.65±0.079	5.48±0.052	0.77 ±0.037
Green bean plug	12.01 ± .115	1.71± .082	4.2 ± .065	.97 ± .046
Potato agar	11.87 ± .118	1.75± .083	5.3 ± .03	.44 ± .021
Lactose agar	13.53 ± .103	1.53± .073	4.48± .04	.61 ± .029
Oat agar	11.98 ± .078	1.16± .055	5.23± .127	1.88 ± .090
STRAIN 298				
Corn meal agar	11.036±0.176	2.61±0.124	3.34±0.056	0.836±0.040
Green bean plug	14.79 ± .094	1.40± .067	4.68± .014	.21 ± .010
Potato agar	12.96 ± .144	2.14± .102	4.56± .0188	.28 ± .013
Lactose agar	13.17 ± .126	1.88± .090	4.54± .061	.91 ± .043
Oat agar	12.98 ± .078	1.16± .055	5.56± .064	.95 ± .045
STRAIN 429				
Corn meal agar	13.03 ±0.138	2.05±0.098	3.94±0.036	0.53 ±0.025
Green bean plug	14.75 ± .095	1.42± .068	3.26± .077	1.15 ± .055
Potato agar	13.07 ± .125	1.87± .089	3.99± .122	1.81 ± .086
Lactose agar	12.75 ± .101	1.52± .072	3.75± .047	.70 ± .033
Oat agar	13.76 ± .123	1.81± .086	4.58± .121	1.80 ± .086
STRAIN 651				
Corn meal agar	14.43 ±0.115	1.71±0.082	4.49±0.013	0.19 ±0.009
Green bean plug	17.23 ± .110	1.64± .078	4.52± .035	.52 ± .025
Potato agar	15.11 ± .115	1.7 ± .081	5.12± .017	.25 ± .012
Lactose agar	15.67 ± .121	1.8 ± .086	4.44± .042	.62 ± .030
Oat agar	15.06 ± .113	1.67± .080	5.38± .028	.42 ± .020

In the fall of 1917, after the strains had been grown on artificial media for a year, they were again grown in plate cultures. In a few of the strains there appeared some mycelial growth which differed in color from

the rest of the growth in that plate. These mutations usually appeared as wedge-shaped or fanlike areas with the point of origin usually at the center of the culture. Sometimes they occurred more toward the periphery of the culture. (Pl. 86, A, B.)

Mutations occurred in the following strains: 943, 297, 615, 495, 940, 510, 561, 536, 527, and 990. These mutations have remained true to the characteristics manifested by the first culture. Figure 2 will serve to illustrate the manner in which the mutations originated. Since these strains were not progenies from a single spore, it was thought that there might be a possibility of having a mixture of strains.

There are several types of these variations. One type had a white, fluffy mycelium. A second type, where the mycelium was confined in the medium, had abundant spore production on the surface. A third type had varying shades of gray mycelium bearing spores. At first these peculiarities in growth were regarded as modifications due to some environmental factor. However, after these variations were transferred to other culture tubes and the resulting cultures always exhibited the same characteristics, they then were considered as mutations. Therefore, single-spored cultures were made from one of the strains.

SINGLE-SPORED ISOLATIONS

On November 14, 1917, single spores were isolated from culture 990. The spores were taken from oatmeal agar, and a suspension was made in sterilized distilled water. A platinum loop was used to transfer a drop of the suspension to a cover glass. Each cover glass was examined with the microscope, and when a drop contained only one spore the cover glass was dropped into a test tube containing potato agar. Three cultures were thus obtained and were designated as 990A, 990B, and 990C. After the spores had germinated and had produced a mycelium, transfers were made to the five media used in culturing the various strains. The growth characteristics of cultures 990A, 990B, and 990C were identical with those of the original culture 990.

On November 26, 1917, transfers were made from culture 990C to potato agar plates. The resulting growth was composed of black and white mycelia, with abundant production of spores in the center of the culture (Pl. 86,C). On December 12, 1917, transfers were made from the white and black mycelia to potato agar plates from the cultures made November 26, 1917. The plates made from the black mycelium became black with some white mycelium. The plates made from the white mycelium were white, but only slight traces of black growth could be detected. All cultures produced abundant spores.

On January 9, 1918, transfers were again made from the two kinds of cultures obtained in transfers of December 12, 1917, with results similar to the transfers of December 12, with the exception that there was

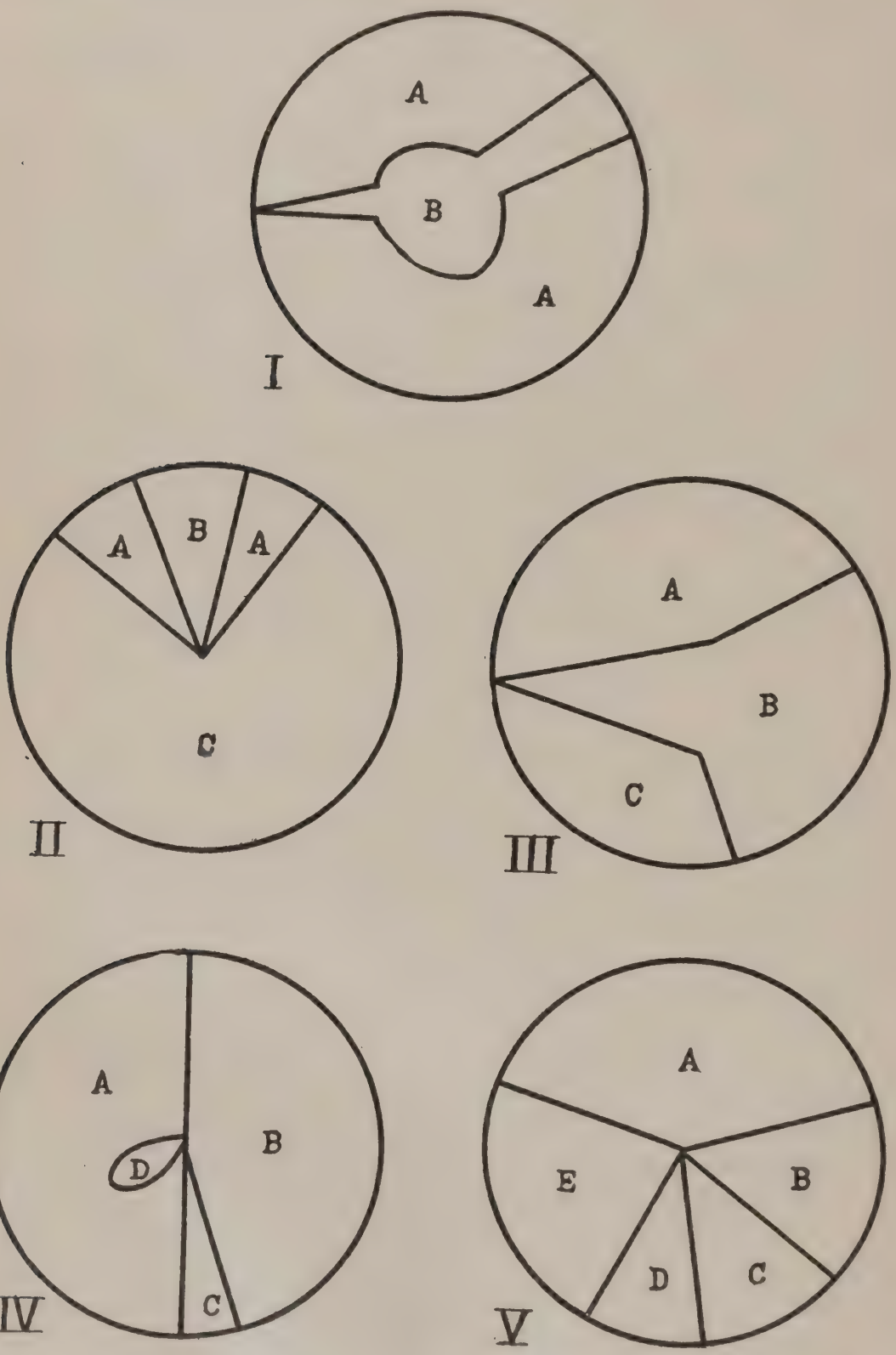


FIG. 2.—I, culture 510: A, greenish black mycelium; B, white mycelium. II, culture 943: A, black mycelium; B, white mycelium; C, mycelium mostly in medium, growth zoned, abundant spore production. III, culture 495: A, black mycelium; B, gray mycelium; C, white mycelium. IV, culture 527: A, gray mycelium; B, greenish black mycelium; C, white mycelium; D, black mycelium. V, culture 940: A, greenish black mycelium; B, white mycelium, some greenish concentric circles; C, black mycelium; D, white mycelium; E, white and black mixed.

practically no black mycelium in the white cultures and but very little white growth in the dark cultures.

Another set of transfers was made on January 24, 1918, from the cultures made January 9, with the result that the white cultures were pure white but the black cultures still produced white hyphae. All plates produced an abundance of spores.

Since the spores are asexual, I wished to determine if they would act like parts of the mycelium when transferred. On January 29, 1918, transfers were made from the spores produced by the white mycelium, and the resulting cultures were pure white, producing many spores. Also spores were transferred from the black and white plates, and the resulting cultures were black with some white hyphae, each culture producing many spores.

The foregoing experiment seems to point to the fact that asexual spores of *Colletotrichum gloeosporioides* act like mycelium when transferred.

The various types obtained by the mutations (fig. 2) are similar to the strains I had in culture. Therefore, one might be led to conclude from the foregoing data that *Colletotrichum gloeosporioides* is constantly giving off new types under natural conditions, as well as in artificial cultures.

SUMMARY

(1) *Colletotrichum gloeosporioides* is a polymorphic species made up of a number of strains.

(2) The various strains when grown on artificial media give distinct cultural characteristics.

(3) Each strain is affected by its environment. The growth characteristics as well as the spore size are varied by the medium on which the strain is grown.

(4) This induced variation may be more or less permanent.

(5) There occur mutations in culture which resemble the strains isolated from the natural environment.

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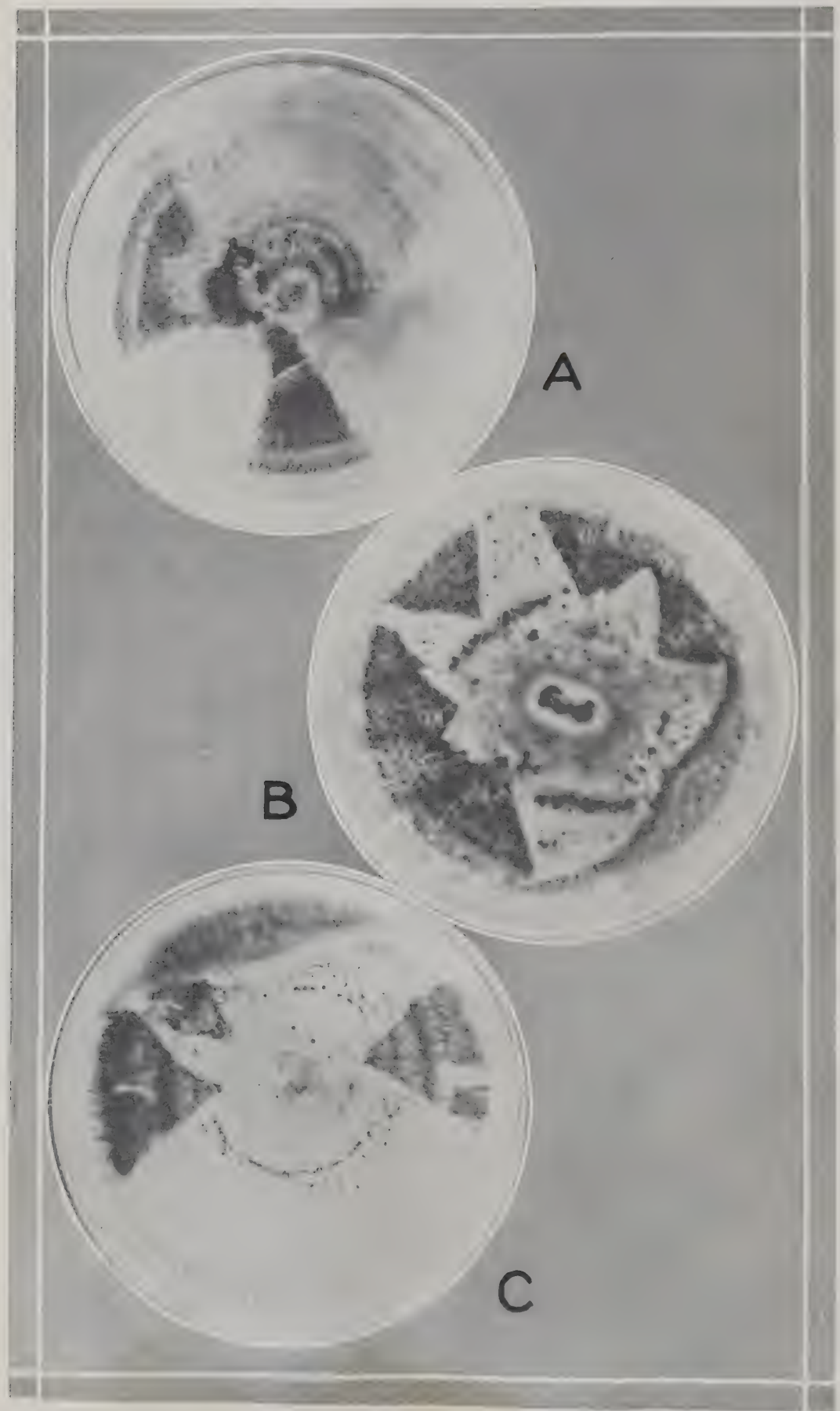

A
B
C

PLATE 86

A, B.—Variation occurring in strain 990. The cultures were not made from a single spore.

C.—Variation occurring in a culture of strain 990 which was made from a single spore.

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A TRANSMISSIBLE MOSAIC DISEASE OF LETTUCE

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During January, 1920, Romaine lettuce (variety Paris White Cos) in a field of several acres at Sanford, Fla., developed a condition very suggestive of a transmissible mosaic disease. The first symptom of disease was a yellowish discoloration along the smaller veins of the younger expanding leaves. This symptom was usually evident for only a few days, giving way to a general yellowish, discolored appearance of the whole plant. All gradations of discoloration occurred, from very marked to conditions not distinguishable with certainty from normal. Close examination usually revealed irregular blotches of an approximately normal green color, which were usually located along the larger leaf veins. The blotching varied from a few barely perceptible green areas on a yellowish leaf to numerous pronounced green spots giving a marked mottled appearance to an occasional plant (Pl. 87, A). The leaves of diseased plants generally seemed to be rather more wrinkled than those of normal plants. Where plants became diseased only after reaching considerable size, the older leaves, which were fully expanded on the first appearance of disease symptoms, frequently continued to appear perfectly normal, while all younger leaves developed the disease symptoms.

At the same time head lettuce (variety Big Boston) in a neighboring field developed a similar diseased condition. The general yellowish, discolored appearance of whole plants was frequently pronounced, but in most cases the blotching was less marked than in the Romaine lettuce, and a decided mottled appearance was never observed.

In general, diseased plants made a stunted growth. In severe cases the plants were decidedly undersized, and occasionally the leaves formed only a rosette, with no indications of a folding together of the tips to form a head. Usually loose heads of poor quality were formed, although all gradations of development, including occasional heads of practically normal size and hardness, occurred. Often plants that showed marked discoloration, mottling, and stunting soon after becoming diseased would later seem to recover in part and to make a more or less normal growth with only slight discoloration and mottling.

Attempts to islolate fungi or bacteria from the apparently healthy plants were unsuccessful, at least in so far as it has not been possible to isolate any organisms capable of producing the disease on reinoculation. Furthermore, examination of the etiolated areas of the diseased plants does not disclose the presence of any recognizable parasite.

Variations parallel in every respect to those described above have been observed frequently by the writer in the mosaic disease of beans.

Approximately 75 per cent, or more, of the plants in these fields became diseased. Frequent observations showed that aphids (*Myzus persicae* Sulz.) were abundant on the lettuce during the time the disease was developing. Similar conditions were observed in April, 1919, when the writer found what appeared to be the same disease in destructive amounts in several fields of head lettuce at Beaufort, S. C., which were at that time nearly ready for harvest. Several growers stated that aphids had been abundant in these fields a few weeks earlier. A disease that seemed identical has also been observed every season for several years in numerous localities in New York State, usually, however, affecting only occasional plants and causing only minor losses. During four seasons (1914–1917) it occurred in practically all fields of lettuce in the vicinity of Rochester, N. Y., where aphids and other insects were usually more or less abundant, while on the same farms lettuce grown during the winter in the greenhouses, where aphids and other insects were held at a minimum by fumigation, was usually entirely free from the disease.

In order to follow up experimentally these observations, which suggested a relation between the mosaic disease and aphids, several insect cages were constructed of cheesecloth, which were large enough to permit the growing of several lettuce plants under each. Lettuce of both the Big Boston and Paris White Cos varieties was grown from seed under the cages in the field at Sanford, Fla., during the winter season of 1920, particular care being exercised to prevent any aphids from reaching the plants except when intentionally placed on them. *Myzus persicae* Sulz. was used in all the experiments.

On February 10 two aphids collected from several mosaic lettuce plants were placed on each of 25 small healthy lettuce plants under an insect cage. When these were examined, on March 8, there were 7 mosaic and 5 healthy Paris White Cos plants and 5 mosaic and 8 healthy Big Boston plants. Twenty-five plants grown under an adjacent control cage, under conditions comparable in every respect except that no aphids had been placed on them, were all healthy, with the exception of one mosaic plant. The plants were still small, having made slow growth on account of cool weather. There were no aphids in the control cage. In the aphid cage there were at least a few aphids on each plant, but they were apparently not numerous enough to interfere materially with normal growth.

Six aphids from a colony on mosaic lettuce plants under a cage were transferred on March 15 to each of 16 healthy, rapidly growing lettuce plants under an insect cage. On March 27 several of these plants showed the first symptom of the mosaic disease, as previously described, and there were several aphids on each plant. On this date all aphids were destroyed by drenching the plants with "Black Leaf 40" solution. On March 31 there were 4 mosaic and 4 healthy Paris White Cos plants and 3 mosaic and 5 healthy Big Boston plants. Sixteen comparable control plants under an adjacent cage were all healthy. On April 15 there were 6 mosaic and 2 healthy Paris White Cos plants and 5 mosaic and 3 healthy Big Boston plants. All the 16 control plants were still healthy. Both cages were free from aphids.

On March 22 three sets of comparable healthy, rapidly growing lettuce plants under three insect cages were treated as follows: Ten aphids obtained from the same colony on mosaic lettuce from which the aphids in the preceding experiment were secured were placed on each plant in cage No. 1. Ten aphids that had presumably never fed on lettuce were collected from a potato field and placed on each plant in cage No. 2. Cage No. 3 was left without aphids, as a control. The first symptom of the mosaic disease was evident on 2 plants in cage No. 1 on March 30 (Pl. 87, B). On April 14 all 4 Big Boston plants and 3 of the 5 Paris White Cos plants in cage No. 1 showed the mosaic disease, while the 9 comparable plants in each of cages No. 2 and 3 were apparently healthy. Aphids were abundant in cages No. 1 and 2 and were lacking in cage No. 3.

CONCLUSION

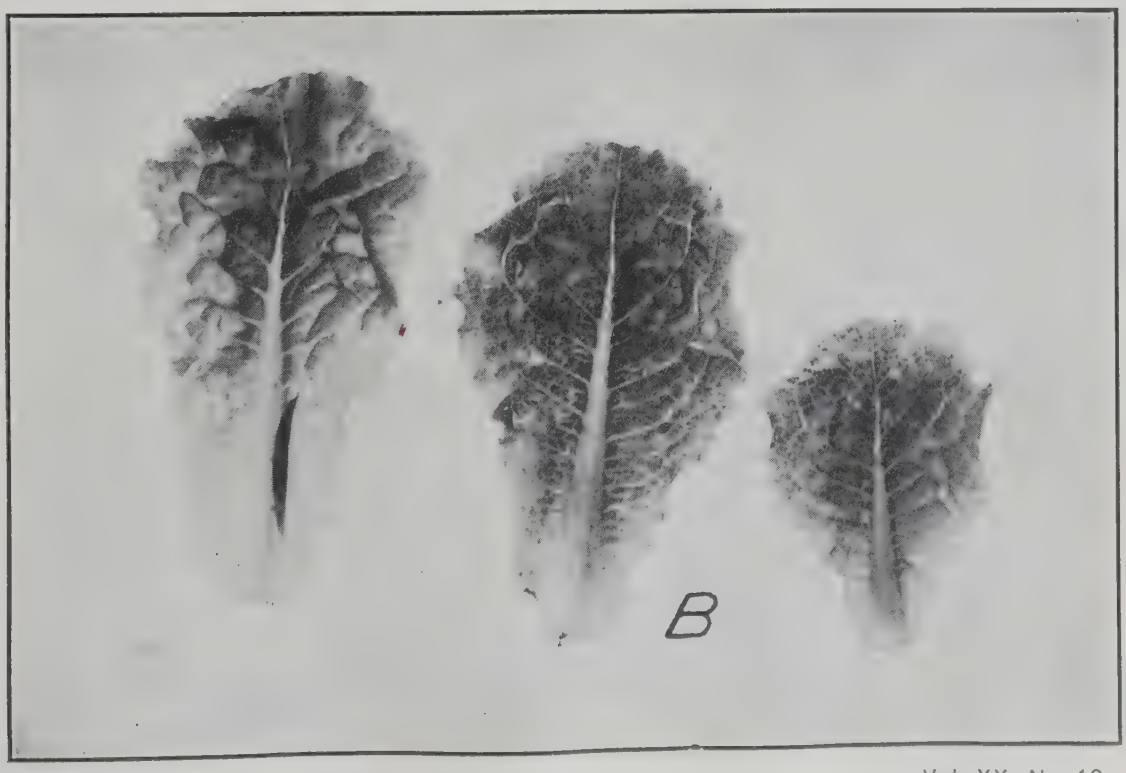
There occurs at Sanford, Fla., a serious infectious disease of lettuce, apparently caused by a parasite not capable of isolation through ordinary microbiological or bacteriological technic. The disease has been transmitted experimentally from diseased plants to healthy plants by means of aphids, particularly the species *Myzus persicae* Sulz. From the symptoms and general character of the disease, it should undoubtedly be recognized as a true mosaic disease of lettuce.

PLATE 87

A.—Leaves of Romaine lettuce. Leaf in center from healthy plant; two others from mosaic plants, one showing pronounced type of mottling and the other general yellowish discoloration with few green blotches along larger veins.

B.—Young expanding leaves of head lettuce from experiment started March 22. Leaf on left from healthy plant; two others from plant in early stage of the mosaic disease, showing yellowish discoloration along smaller leaf veins.


A


B

LECONTE'S SAWFLY,[1] AN ENEMY OF YOUNG PINES

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INTRODUCTION

The following paper on Leconte's sawfly, *Neodiprion lecontei* (Fitch),[1] consists of a detailed description of the various phases of this insect and summarizes the notes on the life and seasonal history. A few notes on the economic importance and the means of control are added.[2]

In describing the larva special care has been taken, and such new terms as have been introduced are carefully explained and illustrated. It is believed that by the introduction of these terms it has been possible to give a more nearly accurate description of the larva and that this terminology will aid in the preparation of descriptions of larvæ belonging to allied groups. The terminology here used is the same as that applied to *Pteronidea ribesii* (Scopoli), *Neodiprion lecontei*, and other sawfly larvæ in a paper ready for publication, and for the reasons therein contained and to avoid possible confusion it seems advisable to continue the use of the same letters to designate the same body areas.

Because of the feeding habits of the larva, Leconte's sawfly is an important enemy to young pine trees in the eastern part of the United States. It is especially injurious to nursery stock. While this paper deals briefly with all of the phases of the insect, more detailed accounts of its life and seasonal history, the damage done, and the means of control have been reserved for future publications of a less technical nature.

DESCRIPTIONS

ADULTS

FEMALE (PL. 88, A)

Length of female 6 to 9.5 mm. Labrum narrowly rounded apically, the surface shining and slightly concave; clypeus broadly subangulately emarginate, apical margin broadly depressed, the basal part convex, with small, poorly-defined punctures; supraclypeal area flattened; antennal foveæ large, shallow, connected with the deep supraclypeal foveæ; lateral foveæ large, circular, deep; middle foveæ and ocellar basin shallow, poorly defined; postocellar area usually well defined, convex, wider

[1] Order Hymenoptera, suborder Chalastogastra, family Tenthredinidae, subfamily Diprioninae.

[2] All the rearing and experimental work on which this paper is based was carried on in the insectaries and nurseries of the Eastern Field Station of Forest Insect Investigations, Bureau of Entomology, located at East Falls Church, Va. The work has been done under the direction of Mr. S. A. Rohwer, specialist in Forest Hymenoptera, and the author is indebted to him for the descriptions of the adults, helpful suggestions, and many of the observations here recorded. Plate 88 was drawn by Miss Mary Carmody, Plate 92 was photographed by H. B. Kirk, and Plates 89 to 91 were drawn by the author.

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posteriorly, somewhat impressed medianly, about two and one-half times as wide as the cephalo-caudad length; postocellar line distinctly shorter than the ocellocular line; antennæ robust, normally 19-jointed but varying from 18- to 21-jointed, apical joints a little more than twice as wide as long, joints 3 and 4 subequal, the basal rami more slender than the apical ones, pedicellum much wider than long; head dulled, with scattered shallow punctures; mesonotum shining, with separate distinct punctures, anteriorly the punctures closer; scutellum with somewhat larger punctures; mesepisternum punctato-reticulate; first parapteron depressed anteriorly and ventrally omitting the depressed area the outline forming an equilateral triangle; tergites, except the ventral aspect, polished, impunctate; last sternite broadly, arcuately emarginate; pad-like apical ventral portion of the sheath a little over four times as long as wide and fitting close to the median ridge of sheath; venation normal. Head, prothorax, and mesothorax rufo-ferruginous; mesosternum blackish to ferruginous; greater part of the mesepisternum sometimes pale ferruginous; metathorax and abdomen black, ventral aspect of tergites whitish, nates and sheath rufo-ferruginous, venter black or in part ferruginous. Legs ferruginous, part of femora and bases of coxæ blackish; bases of tibiæ and basitarsi whitish; occasionally the tibiæ are all whitish. Wings vitreous, subhyaline; venation dark brown. Antennæ black.

MALE (PL. 88, B)

Length 5 to 6.5 mm. Labrum polished, the apical margin rather broadly rounded, clypeus with the apical margin very gently arcuately emarginate, not depressed, the surface sparsely punctured; lateral foveæ practically wanting, other foveæ as in female; ocellar basin represented by a glabrous impression; postocellar area well-defined, subconvex, not impressed, postocellar furrow arcuate; postocellar line slightly shorter than ocellocular line; head with large punctures, those on the front closer, those on the vertex and occiput more widely separated; antennæ 19-jointed; mesonotum with small separate punctures, those of the scutellum rather larger; mesepimeron punctato-reticulate; hypopygidium broadly rounded apically, exceeding the genitalia. Black; labrum pallid; apices of mandibles piceous; legs below trochanters and middle of venter reddish yellow. Wings hyaline, iridescent; venation pale brown.

EGG

Egg 0.25 mm. long by 0.5 mm. broad; envelope very thin, whitish, smooth, shining, translucent, and oval in outline.

LARVA (SIXTH INSTAR) [1]

The following description is prepared from apparently full-grown larvæ from alcohol, approximating 21 mm. in length (Pl. 89, A).

[1] In the description of sawfly larvæ, both structurally and for color, it is necessary that particular areas and regions of a segment or body wall be designated and that the designations adopted be applicable to both the thorax and abdomen of the larva in all its stages. Further, the method, or system, should permit by addition, elimination, change in shape, armature, and spotting of folds, areas, or regions, the comparison with other larvæ, and at the same time should avoid possible confusion of meaning. The following is a suggestion for such a terminology and is the one used in the succeeding pages.

An intermediate (second to eighth, inclusive) abdominal segment of *Neodiprion lecontei* (Pl. 91, B, E) consists of tergum, pleurum, and sternum and begins with the transverse tergal fold immediately preceding that above the spiracle.

The tergum is composed of six transverse folds which are considered as representing four primary divisions (A, B, C, D), with one, the third, twice subdivided ($C^{1,2,3}$).

The pleurum is divided into three folds—the dorsal anterior one here called the preepipleurite, the posterior one called the postepipleurite, and a ventral one called the hypopleurite—and two areas, one containing the spiracle and the other, armed with a few spines, posterior to and adjoining that containing the spiracle. The area containing the spiracle is at the lower extremity of fold B immediately above the preepipleurite

HEAD (PL. 90, A–E)

STRUCTURAL CHARACTERS.—The dimensions of the head are 2.33 mm. in height (dorsad-ventrad) by 1.75 mm. broad. The capsule (Pl. 90, B, C) is of thin chitin with two openings, the occipital foramen in the posterior wall where the head joins the thorax and the buccal foramen in the venter where the pharynx, mandibles, etc., are situated. The head consists of the following sclerites, areas, and organs: Epicranium, eyes, antennæ, frons, adfrons, pleurostoma, hypostoma, clypeus, labrum,

---

and is termed the spiracular area, while the second area, that posterior to the above and armed with few spines, is below folds C 1, 2, 3 and is termed the postspiracular area.

The sternum consists of two transverse folds before the hypopleurites, one between and one behind them. The hypopleurites bear the uropods.

These segmental divisions are all rather well defined externally by infoldings of the skin or body wall (Pl. 89, B; 91, D, E), which serve to bear the attachments of certain muscles. These muscles are of considerable value in defining the folds but are not discussed here in detail, since they would require much comparison of forms, bring matter irrelevant to the subject at hand into the paper, and can better be treated fully in a separate paper after further study. It should be said, however, that the studies made thus far seem to bear out the foregoing conclusions and to offer an excellent method by which to limit segments and segment subdivisions and check up homology of the areas, abdomen to thorax, species to species, and larva to adult.

The interpretation of the segmental composition and terminology outlined above is applied to the thorax (Pl. 91, A, D) in the following way: Each of the three thoracic segments (prothorax, mesothorax, and metathorax) is 4-annulate tergally, and the annulations when viewed with reference to ornamentation, shape, position, and relation with one another homologize in order with the primary divisions (A, B, C, and D) of the abdomen, the third, C, not being subdivided.

The pleurum is distinctly divided into four lobes, preepipleurite, postepipleurite, prehypopleurite, and posthypopleurite, in all three segments; and the postspiracular area is present, in approximately its relative abdominal position, in the mesothoracic and metathoracic segments, despite the absence or displacement of the spiracle.

The sternum consists of three small, rather indistinct folds anterior to the leg's basal attachment to prehypopleurite and posthypopleurite.

Further, the transverse circumference of the larva is divided into longitudinal areas of about equal width, (Pl. 91, F).

TERGUM OR DORSUM

The tergum or dorsum in the present paper is intended to designate that portion of the larva which is dorsad of the spiracular and postspiracular areas and which is divided into transverse folds or annulets A, B, C, and D in the thorax, and A, B, C 1, 2, 3 and D in the abdomen.

I^a.—Middorsal, a single longitudinal midtergal line.

I.—Dorsal, a pair of longitudinal tergal regions, one to either side of the middorsal line.

II.—Subdorsal, a pair of longitudinal regions, one to each side of the dorsal regions.

III.—Laterodorsal, longitudinal regions, laterad of subdorsal regions.

IV.—Supraspiracular, longitudinal regions, laterad of latero-dorsal regions.

PLEURUM OR LATUS

The pleurum or latus designates that portion of the larva between tergum and sternum.

V.—Spiracular, longitudinal regions, one to each side of the larva and ventrad of the supraspiracular regions, with the abdominal spiracle situated therein in most sawfly larvæ, including *Neodiprion lecontei*.

VI.—Epipleural, longitudinal regions ventrad of spiracular.

VII.—Pleural, longitudinal regions ventrad of epipleural.

VIII.—Hypopleural or lateroventral, paired longitudinal regions, in which are situated the hypopleurites, one to either side of the sternum and ventrad of the pleural regions.

STERNUM OR VENTER

The sternum or venter designates that portion of the larva beneath the body between the uropods. The ventrad projection of the uropods places them with reference to the position they occupy in relation to other structures in the adventral longitudinal areas.

IX.—Adventral, paired longitudinal regions containing the uropods, one protruding from each hypopleurite.

X.—Ventral, a pair of longitudinal sternal regions.

X^a—Midventral, a single, midsternal, longitudinal line.

epipharynx, tentorium (arms and bridge), hypopharynx, maxillæ, labium, and mandibles.

The epicranium is the largest area of the head, extending from the dorsal margins of the frons and the lateral margins of the adfrons on the anterior wall of the head to the dorsal margin of the occipital foramen and the lateral margins of the hypostoma on the posterior wall. The epicranium is divided dorsally by a rather faint, median line, the epicranial suture (Pl. 90, G), from the dorsal angle of the frons to the occipital foramen, and has a pair of slight, parallel seams beginning near the lateral extremities of the occipital foramen and extending a short distance dorsally. It is moderately spined generally but has concentrations of spines in the areas about the antennæ, eyes, and pleurostomata. The eyes (Pl. 90, A, D) are a single simple pair, one occurring near each of the lateral extremities of the head and slightly below a line drawn through the dorsad-laterad angles of the frons. The antennæ (Pl. 90, F) are paired and occur one each about midway between each eye and the nearest portion of the pleurostoma. They consist of an elongate projecting cone anteriorly and two flat, floating pieces beyond, one of which is usually faintly connected with a narrow band running forward around the cone. The frons (Pl. 90, G) is an inverted, somewhat shield-shaped area and has for its dorsal margin an angle projecting into the epicranium with its apex at about the height of the head's greatest width. Its lateral margins are nearly parallel and about equal in length to the distance of their separation, while the ventral margin is moderately concave. This sclerite is spined according to a rather regular pattern, but the number of spines and their position vary somewhat. The adfrons (Pl. 90, G) consists of an elongate area of thick chitin situated laterad of the frons and separating it from the epicranium. In outline each adfrons is somewhat triangular and supports the dorsal attachment of a tentorial arm and the dorsal or anterior condyle for the mandible. The pleurostomata (Pl. 90, B) are the thickened lateral margins of the epicranium which extend in an arc around the base of each mandible and support at their anterior and posterior extremities the points of articulation of each mandible. The hypostoma (Pl. 90, B) is a centrally narrowing bridge with its dorsal margin formed by the somewhat angular lower rim of the occipital foramen, its ventral margin formed by the slightly curved posterior rim of the buccal foramen, and its lateral limits defined by the slightly curved and thickened ridges running from the lateral extremities of the occipital foramen to the ventral or posterior fossæ for the mandibles. The clypeus (Pl. 90, B) is a dorsally chitinous, ventrally membranous area immediately below the frons and connecting it with the labrum. It is armed with two pairs of spines arranged to form a transverse row. These pairs are separated from each other about two and a half times the distance between the individuals constituting the pair. The labrum (Pl. 90, I) is slightly bilobed

or rounded laterally and subapically but has a median apical concavity and is ornamented with a transverse row of two pairs of spines. These two pairs are slightly farther apart than are the two spines composing each pair. The epipharynx (Pl. 90, E, I) is a thin skin, armed to each side apically, or under each lobe of the labrum, with a series of inwardly diminishing, opposed setæ or blades, lacking symmetry, which often vary somewhat in number and arrangement. The tentorial arms (Pl. 90, B, C) are a pair of supports or struts diverging to the widely separated pair of adfrontal triangles from the tentorial bridge (Pl. 90, B, C), which is a thickened central attachment of the hypostoma. The hypopharynx (Pl. 90, E, J), or floor of the mouth, rests between and beyond the paired maxillary laciniæ and is a thin membrane, minutely ornamented. Each maxilla (Pl. 90, J–N) is composed of cardo, stipes, palpifer, 4-jointed palpus, galea, and lacinia. The labium (Pl. 90, J, K, O) is composed of submentum (or mentum and submentum fused), mentum (or labial stipes), ligula, and, to each side of the latter and attached basally to the mentum (or labial stipes), a palpiger surmounted by 2-jointed palpus. The mandibles (Pl. 90, H) are 5-toothed.

Color.—The head capsule is orange-brown, excepting the spots surrounding the eyes, which are black, and a part of the clypeus, which is dark brown. The labrum is pale brown with its entire margin darkened, the chitin of the maxillæ and labium is brown to blackish, while the epipharynx, hypopharynx, and ligula are pale white with their armatures pale brown.

THORAX

Structural characters.—The prothorax (Pl. 91, A) when examined exteriorly and in its normal position appears to consist dorsally of but two or three annulets, C and D always and B sometimes. This is due to the constriction of the anterior circumference of the segment in its connection with the head. An examination of the skin infoldings (Pl. 91, D), however, will reveal all four of the primary divisions. On the posterior margin of the segment, but caudad-ventrad of B, which is always distinct supraspiracularly, there is a large, rather elongate area in which the large thoracic spiracle is situated. Ventrad of B and anterior to this spiracular area is the preepipleurite; below the preepipleurite and the spiracular area is the postepipleurite; and under the latter comes the posthypopleurite, anterior to which, and rather strongly chitinized, is the prehypopleurite. The prehypopleurite and posthypopleurite support the 4-jointed legs. That part of the venter not occupied by the prehypopleurite and posthypopleurite is divided by three transverse folds into four annulations, the first annulation with a pair of latero-ventral, chitinized areas, extending one from the base of each leg forward to the occipital foramen, called neck plates. B supraspiracularly, C, preepipleurite, postepipleurite, prehypopleurite, posthypopleurite, the leg joints, and the second and third sternal folds are armed with spines.

The mesothorax (Pl. 91, A, D) is not constricted in circumference anteriorly and is readily seen to be composed of the four primary tergal annulets, a small fold ventrad of A and anterior to the preepipleurite, the postspiracular area, preepipleurite and postepipleurite, prehypopleurite and posthypopleurite, 4-jointed legs, and four transverse sternal folds. A, B, C, preepipleurite, postepipleurite, prehypopleurite, posthypopleurite, leg joints, and third and fourth sternal folds are armed with spines.

The metathorax (Pl. 91, A, D) is similar to the mesothorax, except that the small fold anterior to the preepipleurite and ventrad of A is larger and bears hidden on its posterior surface an exceptionally small spiracle.

COLOR.—The prothorax is whitish with the following exceptions: A supraspiracular black spot on B; black prehypopleurite and leg joints; and a pair of black sternal neck plates.

The mesothorax is whitish with the following exceptions: A subdorsal black spot on A, B, and C; a spiracular and supraspiracular black spot on B and C and the postspiracular area; a black preepipleural spot; and black prehypopleurite and leg joints.

The metathorax is similar to the mesothorax.

ABDOMEN

STRUCTURAL CHARACTERS.—In an intermediate (second to eighth, inclusive) abdominal segment (Pl. 89, B; Pl. 91, B, E) the tergum consists of six transverse folds (A, B, $C^{1, 2, 3}$ and D). The pleurum is divided into preepipleurite, postepipleurite, hypopleurite, spiracular area ventrad of B and bearing the spiracle, and postspiracular area posterior to the spiracular area and below $C^{1, 2, 3}$. The sternum is composed of two transverse folds before the hypopleurite and one behind it. The uropods project from the hypopleurites. Annulets A, B, and C^2, postspiracular area, preepipleurite, and postepipleurite are armed with spines.

The first and ninth abdominal segments are similar (Pl. 91, C) but lack a well-developed hypopleurite and uropod on venter and have four transverse sternal folds.

The tenth abdominal, or anal segment (Pl. 91, C) consists tergally of a large undivided area termed the epiproct, or anal plate; pleurally, of a somewhat triangular fold situated in the anterior portion of the segment similar to the preepipleurite (the anal opening occurring transversely across the apex of the segment); and sternally, of the postpedes, the area from which they spring, and the postcallus below the anus. All folds and areas, except the postpedes, are armed with spines. The area around the base of the postpedes is, however, but slightly spined or haired.

COLOR.—The intermediate (or second to eighth, inclusive) abdominal segments are whitish, with the following exceptions: A subdorsal black spot occurring and diminishing posteriorly on A, B, C^1, and C^2; a supra-

spiracular black spot on B, C^1, C^2, and the dorsad extremity of post-spiracular area; a black spot on preepipleurite; and sometimes a small blackish spot on postepipleurite. The first and ninth abdominal segments are similar but have the preepipleural spot smaller and the post-epipleural spot almost always absent. The tenth abdominal, or anal, segment is white but with the epiproct black.

LARVAL INSTARS

The larval life of sawflies of the group to which this species belongs is divided into two distinct periods by a change of objective. The form and color of the larvæ differ considerably in these two periods. In the first period the larvæ are active and, as they devote most of their energy to feeding, change rapidly in size. There are usually six molts. In the second period the larva is more contracted, less active, and devotes its energies to seeking a place for and constructing the cocoon. No feeding is done in this second period and there is no molting. This second period is generally termed the prepupal period, but other American writers have referred to it as the ultimate stage.

These periods, stages, or instars are measured by the hatching of the larvæ from the egg and by the subsequent sheddings or moltings. The larva molts after slightly varying passages of time, the extent of which will be discussed later; and the molting, as a rule, is accomplished by the longitudinal splitting of the prothoracic and mesothoracic skin mid-dorsally, the breaking of the head capsule along the epicranial suture, and the separating of the frons from the epicranium and the adfrontal triangles. Through the opening thus formed the larva in its new skin endeavors to extract itself from the old, and if successful begins feeding anew, leaving the exuvia attached by the anal end to the needle.

The following descriptions of stages and approximate length of each are the summary of notes from numerous rearings of larvæ in quantities, since it has been found that isolation of larvæ not only tends to retard development but often causes death. This method makes impracticable an absolutely accurate account of the time spent by particular larvæ in each stage. The first appearance of shed skins and of what seemed to be a new stage was, however, recorded and was utilized for description and as an index for these approximations.

The larvæ hatch from the eggs with slightly varying periods of incubation and develop at such different rates that following the first molt there are always two and more often three or more stages present at one time. From about the fifth stage a difference in size of the larva, dependent upon sex, becomes noticeable, to confuse further an endeavor to determine stages accurately.

All the stages are similar to the sixth stage, except as noted in the following descriptions. A detailed description of the sixth instar has already been given under the heading "Larva."

FIRST INSTAR

STRUCTURAL CHARACTERS.—The larva increases in length from about 2 mm. at hatching to about 5 mm. at the beginning of the second stage. In proportion the thorax is slightly large for the abdomen, whereas the head is large for the thorax. The body spines are obsolete, and the spiracles are unusually large, having the appearance of being expanded.

COLOR.—The head is brownish with the eye spots, the labial and maxillary chitin, and the apices of the mandibles blackish. The body is unspotted and previous to feeding is entirely yellowish gray, but upon the filling of the alimentary canal it appears green or lead green. The thoracic leg joints are blackish.

SECOND INSTAR

STRUCTURAL CHARACTERS.—The second stage develops in length from about 5 mm. to 7.5 mm. The head is still large but the thorax and abdomen are nearly normal in their relation to each other. The spiracles are now about normal in their appearance, and the spines are becoming fairly distinct.

COLOR.—The head is brownish with the eye spots and the labial and maxillary chitin blackish. The body is unspotted and pale yellow-green, with the spines appearing faintly grayish, and the thoracic legs are blackish.

THIRD INSTAR

STRUCTURAL CHARACTERS.—The larva in the third instar grows in length from 7.5 mm. to 10 mm. The head is still large, wider and higher than the thorax, and the body spines are now prominent.

COLOR.—The head is brownish to brownish black, with the eye spots black but not conspicuous. The labrum, apices of mandibles, and chitin of the labium and maxillæ are brownish black. The thorax and abdomen are pale, usually unspotted, but in some larvæ with very faint gray supraspiracular spots and epiproct. The thoracic leg joints are black.

FOURTH INSTAR

STRUCTURAL CHARACTERS.—The larvæ of the fourth stage lengthen from 10 mm. to 12.5 mm. The head is now about normal size in relation to the body.

COLOR.—Approximately the same as in the sixth stage. The head varies from brownish to orange and the body is normally spotted with gray black.

FIFTH INSTAR

STRUCTURAL CHARACTERS.—The fifth stage increases in length from 14 mm. to 18 mm. Structure as in sixth stage.

COLOR.—Same as the sixth stage except that the head sometimes has more brown and the body markings appear in some instances proportionally larger and a deeper black than in the sixth stage.

SIXTH INSTAR

In this instar the larva grows from 18 mm. to about 22 mm. For characters, see previous detailed descriptions of larva.

PREPUPA

The prepupa, or seventh larval instar, is the nonfeeding, cocoon-spinning stage in which the larvæ search out a suitable place to spend their quiescent period. In size they usually measure from 10.5 mm. for one which has spun a male or small type of cocoon, to 12 mm. for one which has spun an average size large type or female cocoon.

HEAD

STRUCTURAL CHARACTERS.—The head is 2 mm. in height (dorsad-ventrad) by 1.6 mm. broad, and except for being somewhat smaller is similar to that in the preceding, or sixth, stage.

COLOR.—The head is pale whitish, usually grayish across the dorsum above the eyes. The eyes are pale and are placed somewhat dorsad-caudad of the center of the black oval spot surrounding them. The antennal joints are inconspicuous, being yellowish white on a white membrane. The frons, adfrons, clypeus, labrum, labium, and maxillæ are all pale, the heaviest chitin appearing only yellowish white while the mandibles are pale excepting the teeth, which are brownish black.

THORAX

STRUCTURAL CHARACTERS.—The thorax is similar to that of the sixth stage larva.

COLOR.—The thorax is about the same as that of the sixth stage except that the skin is pale white rather than yellowish white, with spots grayish black rather than black; the mesothoracic and metathoracic subdorsal spots are absent on fold C and very faint on B; and the legs are entirely white.

ABDOMEN

STRUCTURAL CHARACTERS.—The abdomen is similar to that of the sixth stage.

COCOON

The cocoon is a tough, single-walled, papery, red-brown cylinder with hemispherical ends. The exterior, which is darker and less glossy than the interior, shows some coarse threads and often has particles of sand or other surroundings adhering to it. The cocoons vary in size for both sex and individuals. In a number examined, the female, or larger cocoons, varied from 9.5 to 11 mm. in length and from 4.5 to 5 mm. in diameter, averaging 10.3 mm. long by 4.6 mm. in diameter. The male, or smaller cocoons, vary from 7 to 7.8 mm. in length and from 3.2 to 3.5 mm. in diameter, averaging 7.5 mm. long by 3.4 mm. in diameter.

PUPA

Little is known of the pupa stage, but without doubt it is of short duration, since pupæ are rarely found when cocoons are cut open, either shortly after being spun or up to the time they are a year old and have practically all produced adults.

The following descriptions are prepared from a female pupa.

STRUCTURAL CHARACTERS.—The pupa is similar to, though somewhat larger and less hardened than the unemerged adults. The flagellum of the antenna varies from 19 to 20 in the number of joints in the specimens counted. The appendages are folded in or toward the venter with the second pair of wings under the first pair which extend caudadventrad. The shed prepupal skin is attached loosely to the apex of the pupa's abdomen.

COLOR.—The pupa is entirely yellowish, the eyes, apices of the mandibles, and antennæ being the first parts to darken with the development of the adult.

UNEMERGED ADULT

The approach of the pupa toward the mature adult is accompanied by a darkening, or coloration, and hardening of the body wall, which before issuance becomes almost complete, and by the shedding or removal of the pupal membrane or skin, by a reduction in size, and by an increase in activity.

The following descriptions are prepared from an unemerged female adult.

STRUCTURAL CHARACTERS.—The unemerged adult is similar to the mature adult, and the shed pupal skin is attached loosely to the apex of the abdomen.

COLOR.—The head is yellowish brown, with the eyes leaden, the antennæ brownish, the apices of the mandibles brown, and the labium and maxillæ yellowish white. The greater part of the thorax is yellow to yellowish white, but some of the posterior sclerites (mesothorax in part and all of the metathorax) are brownish. The wings are nearly completely developed with their veins brownish, and the legs, excepting small portions, are yellowish white. The abdomen has the tergites (except intersegmental skin) blackish with a broad, white, longitudinal band along the spiracles; the pleural line white; the sternites white medially, brownish near pleural line; and the reproductive parts mostly yellowish.

LIFE AND SEASONAL HISTORY

The length of life of a colony, or the time between the depositing of the first egg and emergence of the last adult, may be approximately either 12 or 14 months—12 months when the eggs are laid in the late summer or early fall and 14 months when the eggs are laid in the later spring or early summer. The length of life of a single colony has been given the name "colony period."

From the cocoons of a single colony there are two periods of adult emergence. The first period is termed "brood A," and the second "brood B." When the colony period begins in late spring or early summer, brood A emerges in the late summer and early fall of the same year and brood B emerges in the late summer and early fall of the following year, making the length of the colony period 14 months. When the colony period begins in the late summer, brood A emerges in the spring and early summer of the following year and brood B emerges in the late summer and early fall of the same year as brood A, making the length of the colony period 12 months.

Thus (see year II in fig. 1) we may have adults of brood B of the first colony period, brood B of the second colony period, and brood A of the third colony period existing in the late summer of the same year. In

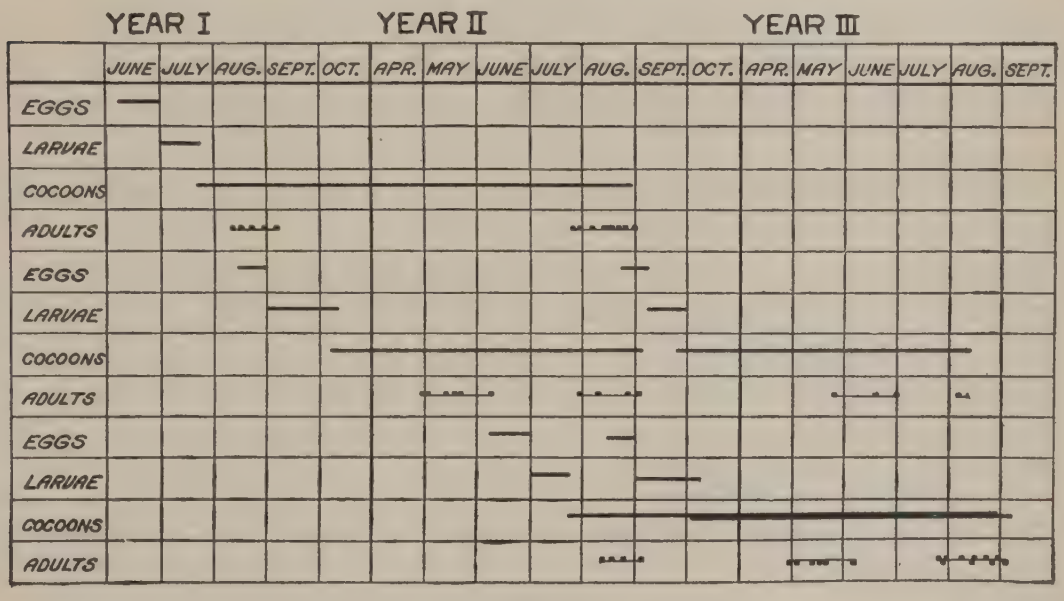


FIG. 1.—Chart showing life and seasonal history of *Neodiprion lecontei* through the active period of three years (November to March omitted, the insect being in the cocoon during this period).

the spring, however, it is possible only to have brood A, but these may be from different colony periods (see year III in fig. 1).

The eggs are laid in a row of slits along one of the serrated edges of the leaf (Pl. 92, B). These slits, the work of the female's saw, are about 1.5 mm. long and 0.8 to 0.9 mm. deep and have an interval between them about equal to their length. They are somewhat shoe-shaped, the opening or slit not entirely covering the pocket, and deepen slightly toward the apex, or toe. These egg punctures are rather conspicuous, appearing yellowish against the green of the undisturbed leaf tissue and becoming brownish with age. Usually the leaves containing eggs die and become noticeable some time after the hatching of the larvæ.

In cage experiments the number of eggs laid by single females varied from 25 to 178, with an average of 82. In six virgin females dissected the number of eggs varied from 58 to 218, with an average of 139, so it is

certain that in these experiments the maximum number of eggs was not obtained. Available data indicate that approximately two-thirds of all the eggs laid produce larvæ.

As a result of there being two periods of adult emergence there are two periods of oviposition and incubation during the year, coincident with those of issuance, the first occurring in the late spring and early summer (particularly May and June) and the second in the late summer and early fall (late July, August, and early September).

The period of incubation as determined by the time elapsing between the laying of the first egg and the hatching of the first larva varies from 13 to 21 days with an average, from six experiments, of 16 days.

For the first 5 or 6 days after oviposition very little change is noted in the eggs, but beginning with the seventh or eighth day a gradual swelling is evident, so that by the ninth day there is a slight separation of the sides of the mouth of the egg pocket. This separation increases until it is 0.5 mm. in breadth shortly before the egg hatches and the larva emerges.

The length of the larval feeding period, from the hatching of the eggs to the appearance of the first prepupa, varies from 25 to 31 days, with an average of 28 days.

During the whole of the feeding period the larvæ are gregarious and show little or no tendency to disperse. If disturbed while feeding they throw back the head and thorax and remain motionless in that attitude, attached to the needle only by the uropods.

The larvæ for the first, second, and third stages eat only the epidermis and the immediately adjoining tissue of the needles. The approximate length of the first stage is 6 days, of the second 5 days, and of the third 5 days. Beginning with the fourth stage and continuing through the sixth, the larvæ eat the whole of the needle and occasionally portions of the tender bark on the young twigs (Pl. 92, A). Field observations on the feeding upon the bark seem to indicate, however, that the species of the tree may have more influence than the amount of foliage available. The bark of the jack pine (*Pinus banksiana*) in Wisconsin and Virginia was usually fed upon, even though there was plenty of foliage available. The approximate length of the fourth stage is 5 days, of the fifth 4 days, and of the sixth 4 days.

Following the larval feeding period comes the prepupal instar, a larval, nonfeeding, cocoon-spinning, quiescent stage. The prepupæ first seek a suitable place and then spin their cocoons. In nature the cocoons have only been found several inches under the surface of the ground under the tree attacked.

After the cocoon is made the insect remains for a comparatively long time as a prepupa, but shortly prior to the time of its emergence it transforms to the pupa and then develops rapidly into the adult stage, which

cuts an end completely, or nearly so, from the cocoon, and issues. It is in the cocoon that this insect passes the winter.

The length of the cocoon period, from its spinning until the issuance of the adult, varies with the character of the colony. If the cocoons are made by the larvæ hatching from eggs laid in early summer (May or June) there will be an emergence, called brood A, in the late summer or early fall of the same year (late July, August, and early September) and a second emergence from cocoons made by larvæ of the same colony, called brood B, in the late summer and early fall of the following year. In such instances the length of the period between the first cocoon and the first adult of brood A varies from 13 to 23 days, averaging 18 days; and the period between the first cocoon and the first adult of brood B varies from 364 to 379 days, averaging 371.

If, however, the cocoons are made by the larvæ hatching from eggs laid in the late summer or early fall the emergence of brood A will not take place until late spring and early summer of the following year, while brood B will emerge in the late summer and early fall of the same year as brood A. In this instance the time elapsing between the making of the first cocoon and the first emergence of brood A varies from 205 to 242 days, averaging 218 days, while that between the making of the first cocoon and the first emergence of brood B varies from 292 to 342 days, averaging 309 days.

The female adults seem to predominate throughout any period of emergence and in a whole colony by the ratio of 3 to 1. Although the females predominate for any given period of emergence or brood in the sense in which it has been used in this paper, it is not unusual to find that at either the beginning or end of the period males will emerge in the majority.

EFFECT OF METEOROLOGICAL CONDITIONS

Eggs laid in late July and early August—that is, during the warmest periods—hatch more quickly than those laid later or earlier in the year. The particular period of the year, however, or the heat has not been proved to be directly responsible for the speed of development, although from temperature readings during the periods of incubation this would seem to be a fact. For example, in June, 1917, when the mean temperature during the incubation period was 71.23° F., with a mean minimum of 60.14°, eggs hatched in 21 days; in mid-August, when the mean temperature during incubation was 74.59°, with a mean minimum of 63.59°, the eggs hatched in 18 days; and in late July and early August, 1917, when the mean temperature of the incubation period was 78.8°, with a mean minimum of 67.69°, eggs hatched in 13 days.

Further, the relation of humidity to development must be considered, and it would seem from our records that high humidity tends to retard incubation. For example, in June, 1917, when the average humidity

during the incubation period was 74.30 per cent, eggs hatched in 21 days; in mid-August, when the average humidity during incubation was 67.80 per cent, eggs hatched in 18 days; and in late July and early August, 1917, when the average humidity of the incubation period was 65.57 per cent, eggs hatched in 13 days.

TABLE I.—*Record of temperature and humidity during incubation period of Neodiprion lecontei*

Date.	Length of incubation period.	Mean maximum temperature.	Mean temperature.	Mean minimum temperature.	Mean relative humidity.
1917.	*Days.*	°*F.*	°*F.*	°*F.*	*Per cent.*
June 9 to 29	21	82.33	71.23	60.14	74.30
July 30 to Aug. 11	13	89.92	78.80	67.69	65.57
Aug. 15 to Sept. 1	18	85.36	74.59	63.83	67.80

It is highly probable that normal development or acceleration is due to the favorable combination or balance of both temperature and humidity and that there are definite limits beyond which heat or moisture would be either insufficient or excessive and result in retardation or death.

Notes on the response of larvæ of this species to meteorological influences are few and somewhat contradictory. The author has observed a decided retardation of activity, feeding, and development, when damp, cold, and cloudy weather occurs in the warm season, and a corresponding acceleration on sunny days. Colonies were found feeding near Falls Church, Va., on November 5, the day being bright but after a heavy frost, while S. A. Rohwer records "nearly full-grown larvæ feeding on the sheltered side of a tree even though it was below freezing and snowing hard," near Trout Lake, Boulder Junction, Wis., on September 21, 1913.

MATING AND COPULATION STUDIES

The females occasionally are, or seem to be, active in finding a mate, but more frequently they appear to resist the attempts to mate offered by the male, sometimes cutting off portions of his antennæ and legs with their mandibles. In those instances where copulation was observed there were no preliminary attentions or courtship. Intercourse takes place with the pair in positions in which their abdomens are opposed. It was observed once that the male arrived in position by crawling over the female from head to posterior end. When his abdomen had reached the end of the female's he swung his under hers. During copulation the wings are held flat against the body; the legs are spread rather far apart, the forelegs projecting anteriorly, the middle legs slightly anteriorly, and the hind legs posteriorly; and the antennæ are usually moved slowly, up and down.

Rohwer[1] gives the following description:

Copulation lasts about 100 seconds and is accomplished by the two individuals facing in opposite directions and the extreme end of the male abdomen being bent at an obtuse angle because of the truncate abdomen of the female. The hypopygidium of the male fits over the knob at the base of the sheath, the harpes grasp the sides of the knob in the manner of a ball and socket joint, while the position occupied by the parapenes, sagittæ, volsellæ, and penis valves, was not observed.

OVIPOSITION STUDIES[2]

After locating a suitable place for ovipositing, the female stands with her legs grasping the needle, her abdomen bent ventrally so that its apex comes in contact with the needle at a point between the mesothoracic and metathoracic tarsi. She seems to start the incision with the lance as well as the lancets by pulling or sliding these away from her along the

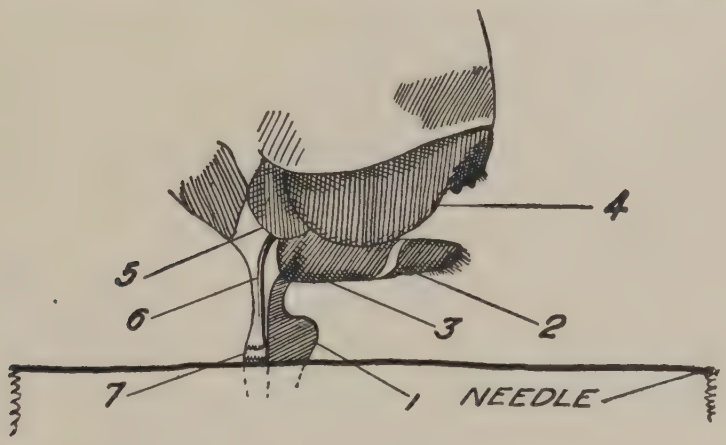


FIG. 2.—Position of end of abdomen of female when ovipositing, showing the various parts and their position: 1, lance; 2, apical part of sheath; 3, basal part of sheath; 4, nates or ninth tergite; 5, eighth sternite; 6, chitinized rods at base of lancet; 7, lancet.

needle in a fashion suggesting an attempt to catch a sharp point or tearing edge in the tissue. After starting the incision she withdraws the lance slightly and appears to use it to guide the lancets and to keep the latter pressed against the front of the cut (fig. 2). After the insertion of the lance and the lancets the female straightens or raises the ventrally bent end of her abdomen, causing the ovipositor to form an abrupt angle with it.

The chitinized basal rods of the lancets run along the chitinized ventral side of the lance and turn into the abdomen towards the ninth tergite. Their up and down motion seems to be controlled by a somewhat side to side movement of the nates, or ninth tergite. The lancets work opposite each other except at withdrawal, when they are worked together up and down and back, following the lance through the arc of the cut they have

[1] ROHWER, S. A. THE MATING HABITS OF SOME SAWFLIES. *In* Proc. Ent. Soc. Wash., v. 17, no. 4, p. 195–198, fig. 1, pl. 22. 1915.
Page 196: Diprion lecontei.

[2] Terminology used here is that adopted in a recent paper (still in proof) by S. A. Rohwer.

just completed. As the ovipositor is removed from the cut the female squats over the freshly made opening and probably at this time deposits the egg. The deposition of the egg could not be seen, but it is believed that the egg does not descend through the ovipositor but that it is dropped in place, leaving the body of the female through the spread bases of the ovipositor, before the ovipositor is completely withdrawn.

The following is an account of the time spent by one female in each of the different steps in the laying of an egg: In scratching the surface of the needle endeavoring to start the incision, she spent 2 minutes and 13 seconds; in working the lance and lancets into the tissue, she spent 22 seconds; in beginning the pocket the female, with her abdomen bent and close to the needle, worked for 27 seconds; and on the remainder of the cutting of the pocket, with her abdomen raised, she worked 1 minute and 49 seconds. The removal of the ovipositor and the deposition of the egg were accomplished in 16 seconds.

PERIODIC APPEARANCE

Leconte's pine sawfly appears and disappears periodically. For several years this species will be very abundant; then for a few years it will become rare. The cause for this periodic disappearance has not been determined, but it seems likely that some factors other than parasitism play an important rôle, because we have no records which give a sufficiently high percentage of parasitism to lead one to believe that this is entirely responsible for a great reduction of the species. Investigation of certain other means of natural control has thrown no light on the subject.

PARTHENOGENESIS

Experiments to determine if this species can reproduce parthenogenetically are inconclusive. In all these experiments only unfertilized females of both emergence periods of brood A were used, and although all of them were failures the information acquired is inadequate to prove that the adults of this brood can not reproduce parthenogenetically. Eight experiments were performed, six of which produced eggs while two failed entirely. In two experiments conducted under especially favorable conditions the eggs hatched but the young larvæ died without molting. It is thus possible to state that females of brood A of this species can and will lay eggs unfertilized and that these unfertilized eggs will hatch, but in no experiments have these larvæ produced adults.

HOSTS

This species appears to have three primary or preferred hosts and a quantity of secondary or possible hosts. The primary hosts as determined by observations in the field and the nursery are: Jack pine (*Pinus banksiana*), which was subject to attack in Vilas and Oneida

Counties in Wisconsin, at Kanawha Station, W. Va., and in the experimental nursery at East Falls Church, Va.; red pine (*P. resinosa*), which was commonly attacked in Vilas and Oneida Counties, Wis., and has been recorded by a correspondent as being attacked at Hyde Park, Dutchess County, N. Y., but which in experiments for oviposition by adults and as food for larvæ conducted in the nursery at East Falls Church, Va., has always led to failures; and scrub pine (*P. virginiana*), which is the native host of this insect through northern Virginia, Maryland, and Pennsylvania.

The secondary or possible hosts can not be ranked as complete hosts capable of supporting the insect through all its various stages or as entirely acceptable to females for oviposition. They have been determined by observation in the field and nursery, from correspondence and literature, and through experimentation. They are white pine (*Pinus strobus*) in Wisconsin and at Reading, Pa.; Scotch pine (*P. sylvestris*) at Reading and Austin, Pa.; loblolly pine (*P. taeda*) Annandale, near Falls Church, Va., and Clinton, La.; shore pine (*P. contorta*) at Kanawha Station, W. Va.; silver pine (*P. monticola*) in the nursery at the Eastern Field Station; mugho pine (*P. mughus*), West Chester, Pa.; *P. eldarica*, Yarrow, Md., chosen in the field and nursery; western yellow pine (*P. ponderosa*), used in experimentation (confining adults in a cage upon the young tree); and longleaf pine (*P. palustris*),[1] Austrian pien (*P. austriaca*),[2] and American larch (*Larix americana*),[3] mentioned in literature and correspondence.

PARASITES

Neodiprion lecontei is subject to attacks by both parasitic insects and a wilt. Four species of hymenopterous and four species of dipterous adults have been reared from the cocoons of this species, but neither egg parasites nor parasites which emerged from uncocooned larvæ have been obtained. The hymenopterous parasites were determined by S. A. Rohwer as *Exenterus diprioni* Rohwer, *Lagorotis diprioni* Rohwer, *L. virginiana* Rohwer, and *Perilampus hyalinus* Say. Of these parasites *L. diprioni* Rohwer is much the most abundant species, and *Perilampus hyalinus* Say is probably a hyperparasite. The dipterous parasites were determined by C. T. Greene as *Phorocera claripennis* Macquart, *Adomonita demylus* Walker, *Neopales maera* Van der Wulp, and *Spathimeitenis spinigera* Townsend.

The wilt of the larvæ was probably a bacterial disease and was found in Wisconsin by S. A. Rohwer, in 1912. The larvæ attacked were readily distinguished by their lack of vigor and their white tracheal system,

[1] Larvæ sent in by a correspondent from Pinehurst, N. C., with the following note. "Eating the pine needle of the longleaf pine in this vicinity."

[2] RILEY, C. V. NINTH ANNUAL REPORT ON THE NOXIOUS, BENEFICIAL, AND OTHER INSECTS OF THE STATE OF MISSOURI, p. 32–33. Jefferson City, Mo. 1877.

[3] "When forced to, defoliate and girdle," in letter from W. D. Barnard, Boulder Junction, Wis.

which was conspicuous early in the disease when the larvæ were yellow and more noticeable later when the larvæ became darkened. The wilt was rather widespread in this locality of infestation, but though it killed a considerable quantity of the larvæ yet its success was limited.

From our notes and rearing records it would seem that none of the insect parasites were abundant enough nor was the wilt sufficiently distributed and infectious to account for the periodic disappearance of this species. It is certain that neither any nor all of these natural checks are sufficiently numerous or effective to admit disregard of the artificial control measures suggested below.

DISTRIBUTION

Neodiprion lecontei was described by Fitch from specimens collected in New York, while Riley and Norton mentioned specimens coming from Ridgewood, N. J. The localities represented in the United States National Museum collection are Baltimore, Md., and Virginia (near the District of Columbia), material collected by Theo. Pergande; and Long Island, N. Y., material collected and reared by H. G. Dyar. The "Guide to Insects of Connecticut" [1] records the sawfly from Middletown, Hampton, and Stamford, for that State. To these localities, through collecting by members of the Bureau of Entomology and correspondents, the following localities have been added (fig. 3):[2]

CONNECTICUT: Cheshire, Deep River, Ellington, New Haven, Norfolk.
DISTRICT OF COLUMBIA: Throughout.
LOUISIANA: Clinton.
MARYLAND: Yarrow, Plummers Island.
MICHIGAN: Remus.
MISSISSIPPI: Orange Grove.
NEW YORK: Hyde Park (Dutchess County).
NORTH CAROLINA: Pinehurst.
PENNSYLVANIA: Austin, Linglestown, Reading, West Chester.
VIRGINIA: Falls Church and vicinity (generally throughout Arlington and Fairfax Counties).
WEST VIRGINIA: Kanawha Station.
WISCONSIN: Generally throughout Oneida and Vilas Counties.

ECONOMIC IMPORTANCE

This species does considerable damage to both natural reproduction and nursery stock by defoliating the trees. Complete or nearly complete defoliation before late summer usually kills that part defoliated;

[1] VIERECK, Henry Lorenz, et al. GUIDE TO THE INSECTS OF CONNECTICUT. PART III. THE HYMENOPTERA, OR WASP-LIKE INSECTS, OF CONNECTICUT. Conn. State Geol. and Nat. Hist. Survey Bul. 22, p. 44. 1916.

[2] Since this manuscript has been prepared this species has been received from the following additional localities:

CONNECTICUT: Hartford.
FLORIDA: Orlando.
NEW HAMPSHIRE: Wonalancet.
PENNSYLVANIA: Clearfield, New Germantown.

and since this insect shows a very decided preference for young trees, and the larvæ often are numerous enough to strip the tree entirely of leaves, many young pines are killed by this work alone. Trees not completely denuded often die because in their weakened condition they are attacked by secondary insect enemies. When there is incomplete defoliation and the tree recovers it is often stunted or misshapen and is of little commercial or ornamental value.

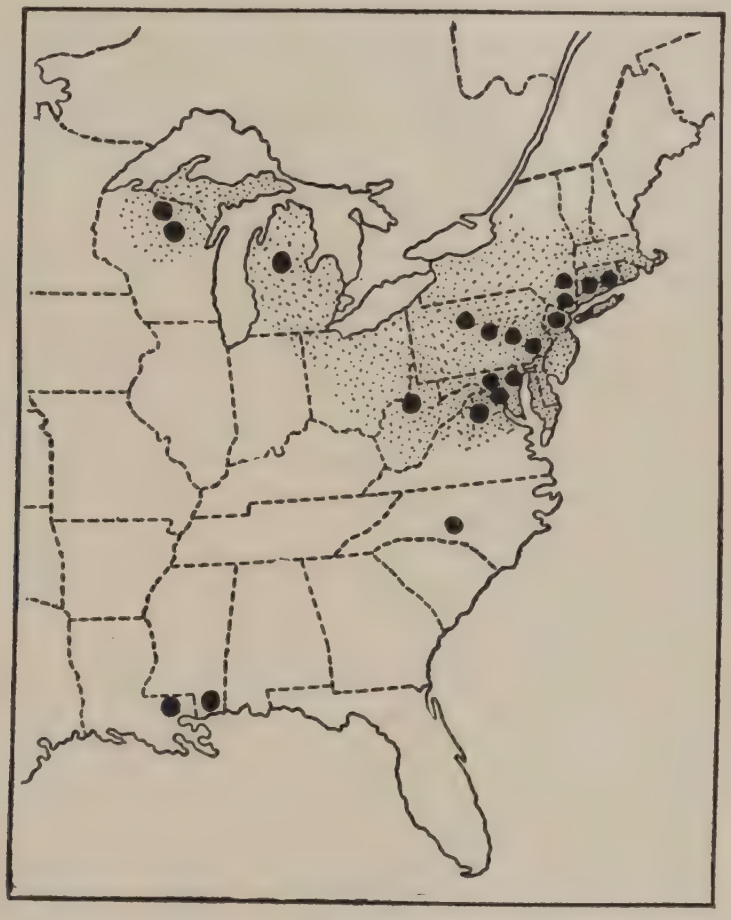


FIG. 3.—Distribution of *Neodiprion lecontei*. The larger dots indicate places from which specimens have actually been received. See also footnote 2, p. 758.

MEANS OF CONTROL

The control of this species depends largely on the extent and location of the infestation. In large areas of either natural or artificial reproduction, control because of its expense can not be generally practiced, but rangers and lumbermen should make it a practice to destroy the colonies of these larvæ whenever they are found. The easiest way is to knock the larvæ from the trees and crush them with the foot.

In nurseries and in parks the control, in case of heavy infestation, can best be attained before the larvæ are full-grown and should consist of thorough spraying. An arsenate of lead spray of 2 pounds of powder to 50 gallons of water (or a ratio of 1 to 12) should be satisfactory. On larvæ which are discovered when young, less than ⅜-inch long, nicotine

sulphate is a fairly satisfactory spray to use; however, because of the resistance of conifers to arsenical sprays and because an arsenical treatment gives more certain results, it is probable that the spray first recommended should be used almost exclusively. In scattered infestations hand picking or knocking the larvæ from the trees and crushing them will be found to be much more economical and at least as effective.

Whenever these insects are observed in any locality and control measures are practiced against them, it is important that the territory be carefully surveyed for the following 14 months, since it is possible that some larvæ may have escaped the treatment and have spun cocoons. This possibility makes watchfulness necessary over the entire colony period of the species in order that an emergence of adults from these cocoons may not reestablish the infestation.

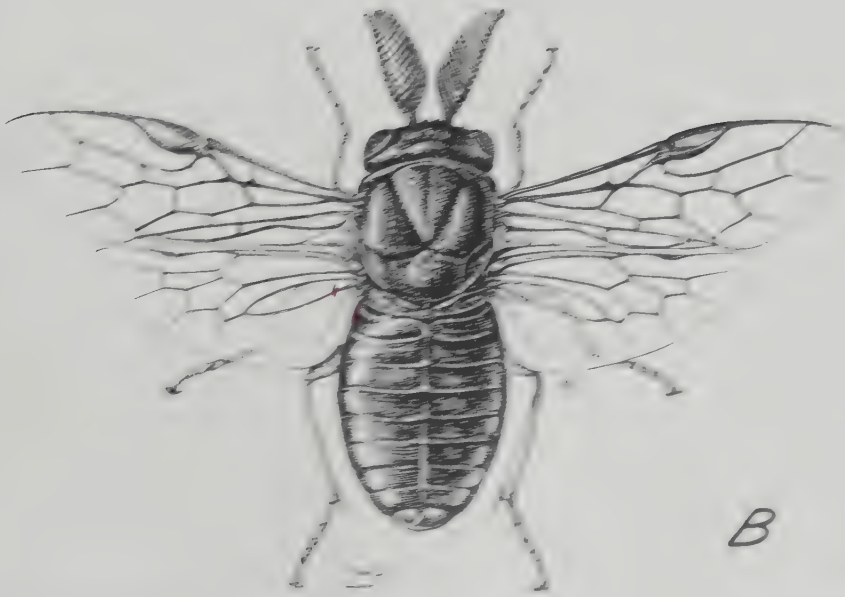
PLATE 88

Neodiprion leconteï

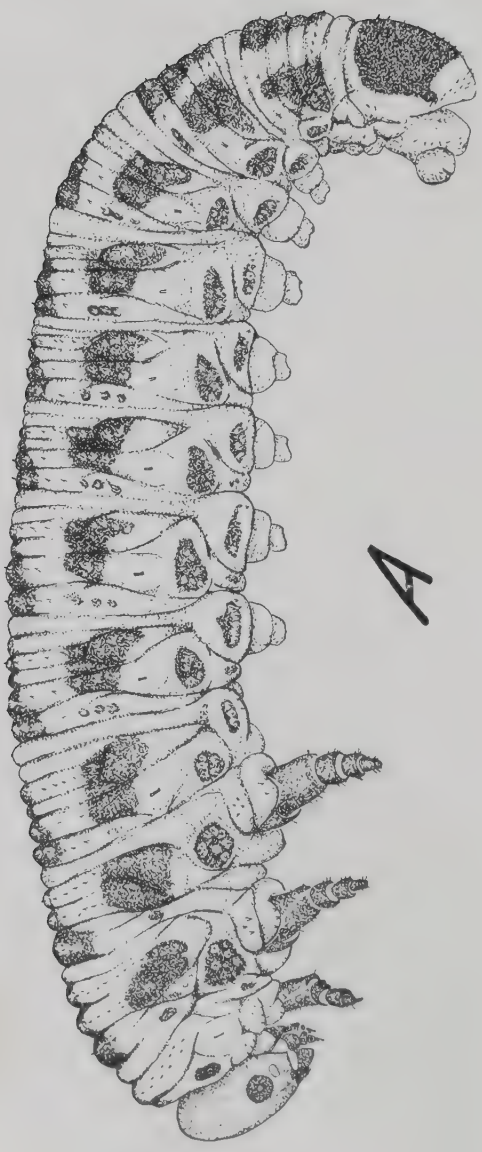
A.—Adult female.
B.—Adult male.



A



B


A

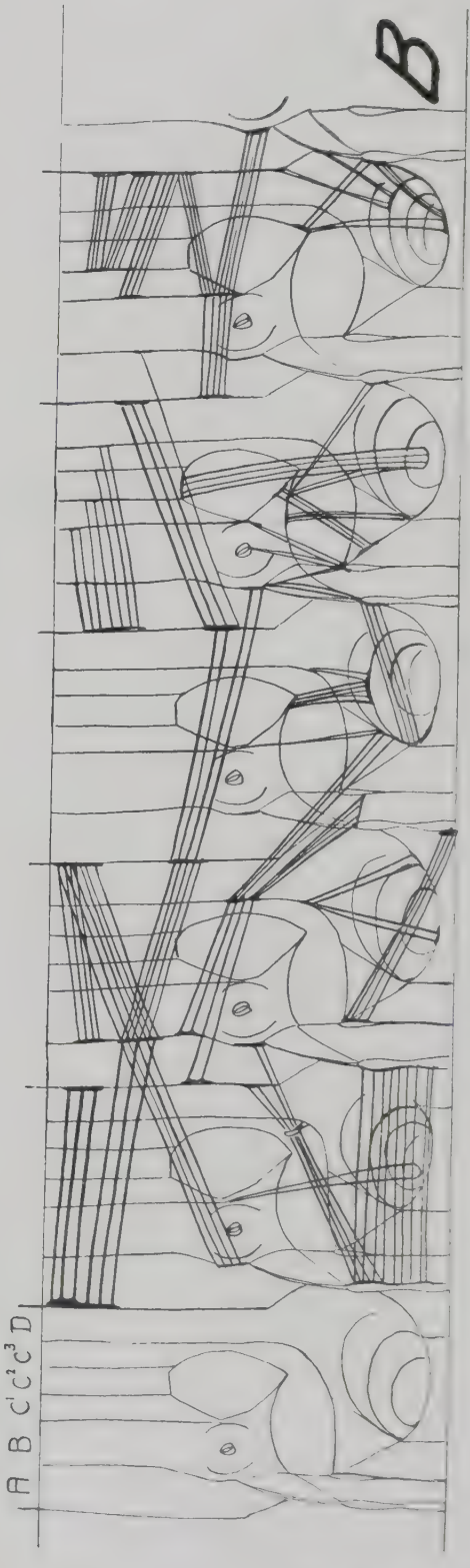

B
A B C¹ C² C³ D

PLATE 89

Neodiprion lecontei:

A.—Larva.

B.—Sixth-stage larva: The muscles of a single abdominal segment distributed over several segments to show their numbers, position, and attachment.

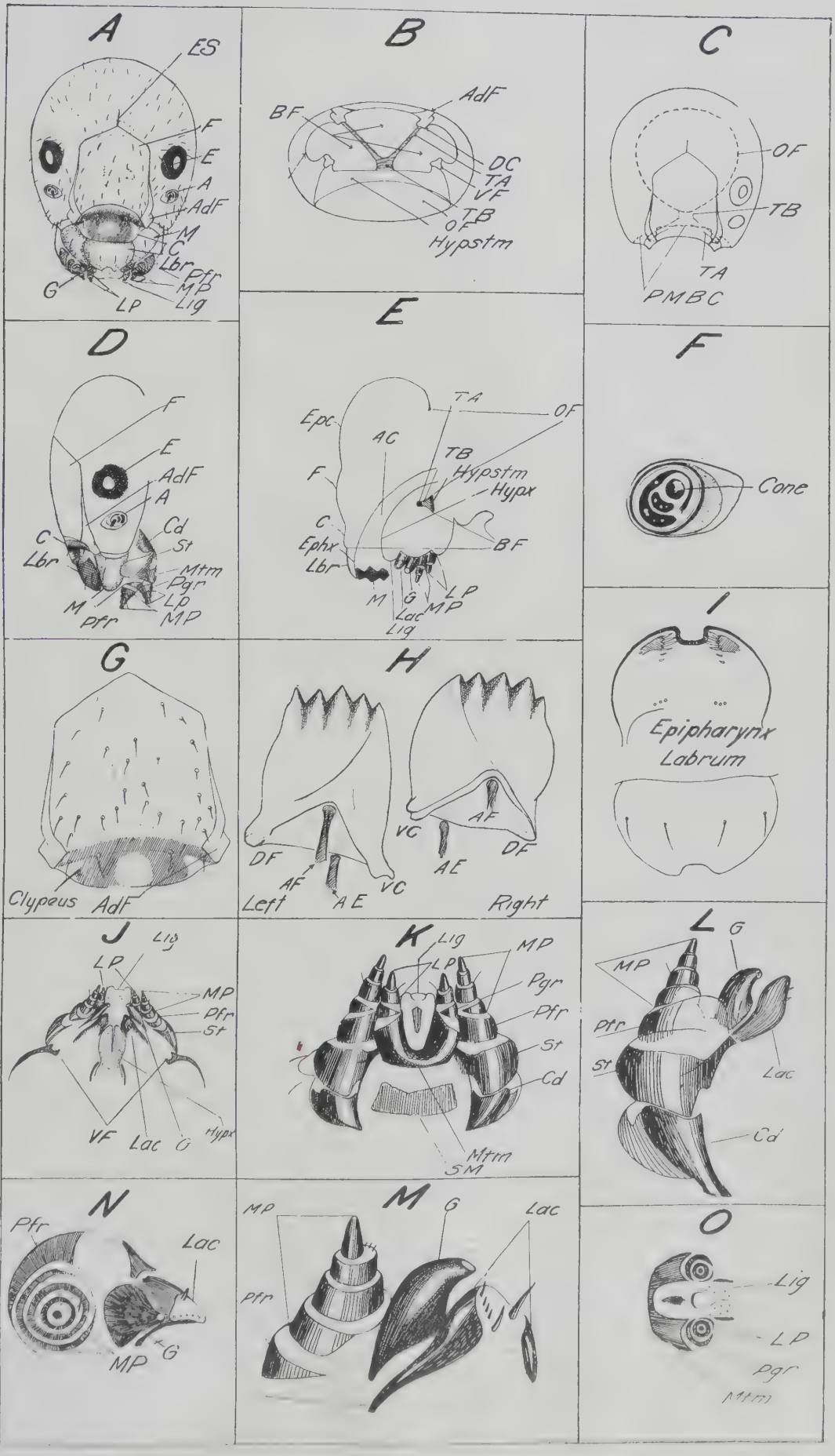
PLATE 90

Neodiprion lecontei: Sixth-stage larva.

A.—Front view of head.
B.—Ventral (or apical) view of head capsule.
C.—Front view of head capsule.
D.—Lateral view of head.
E.—Sagittal section of head.
F.—Antenna.
G.—Frons, adfrons, and clypeus.
H.—Mandibles.
I.—Epipharynx and labrum.
J.—Internal view of hypopharynx, maxillæ, and labium.
K.—External view of maxillæ and labium.
L.—External view of maxillæ.
M.—Interior and apical view of maxilla.
N.—End view of maxilla.
O.—End view of labium.

EXPLANATION OF SYMBOLS

A, antenna.
AC, alimentary canal.
AdF, adfrons.
AE, attachment of extensor muscle.
AF, attachment of flexor muscle.
BF, buccal foramen.
C, clypeus.
Cd, cardo.
DC, dorsal or anterior condyle for mandible.
DF, dorsal or anterior fossa of mandible.
E, eye.
Epc, epicranium.
Ephx, epipharynx.
ES, epicranial suture.
F, frons.
G, galea.
Hypstm, hypostoma.
Hypx, hypopharynx.
Lac, lacinia.
Lbr, labrum.
Lig, ligula.
LP, labial palpi.
M, mandible.
MP, maxillary palpi.
Mtm, mentum.
OF, occipital foramen.
Pfr, palpifer.
Pgr, palpiger.
Plstm, pleurostoma.
PMBC, posterior margin of buccal cavity.
Sm, submentum.
St, stipes.
TA, tentorial arms.
TB, tentorial bridge.
VC, ventral or posterior condyle of mandible.
VF, ventral or posterior fossa for mandible.


A
ES
F
E
A
AdF
M
C
Lbr
Pfr
MP
Lig
G
LP
B
AdF
BF
DC
TA
VF
TB
OF
Hypstm
C
OF
TB
TA
PMBC
D
F
E
AdF
A
Cd
St
C
Lbr
Mtm
Pgr
Lp
M
Pfr
MP
E
TA
OF
Epc
AC
TB
Hypstm
Hypx
F
C
Ephx
BF
Lbr
M
G
LP
Lac
MP
Lig
F
Cone
I
Epipharynx
Labrum
G
Clypeus
AdF
H
DF
AF
VC
Left
AE
VC
AF
DF
AE
Right
J
Lig
LP
MP
Pfr
St
VF
Lac
G
Hypx
K
Lig
LP
MP
Pgr
Pfr
St
Cd
Mtm
SM
L
G
MP
Pfr
St
Lac
Cd
N
Pfr
Lac
MP
G
M
MP
G
Lac
Pfr
O
Lig
LP
Pgr
Mtm

Pro-T
Meso-T
Meta
A B C D A B C D
PSA
SA
Sp
Prep
Psep
NP
Pshyp
Prhyp
Leg Joints
1
2
3
Claw 4
A
THORAX
ABDOMEN
A B C D A B C D A B C D A B
D
A B C C' C'' C''' D
PSA
SA
Sp
Prep
Psep
Hypop
Uropod
B
A B C C' C'' C''' D
10
C
A B C C' C'' C''' D
E
1a
I
II
III
IV
V
VI
VII
VIII
IX
X
Xa
SA
Sp
PSA
Prep
Psep
Hypop
Uropod
F

PLATE 91

Neodiprion lecontei: Sixth-stage larva.

A.—External view of the thorax.

B.—External view of the second and third abdominal segments.

C.—External view of the ninth and tenth abdominal segments.

D.—Internal view of thoracic skin.

E.—Internal view of the skin of the second and third abdominal segments.

F.—Diagrammatic cross section of the abdomen showing the longitudinal areas of the body on its transverse circumference.

EXPLANATION OF SYMBOLS

Hypop, hypopleurite.
NP, neck plate.
Prep, preepipleurite.
Prhyp, prehypopleurite.
PSA, postspiracular area.
Psep, postepipleurite.
Pshyp, posthypopleurite.
SA, spiracular area.
Sp, spiracle.

I^a, middorsal; I, dorsal; II, subdorsal; III, laterodorsal; IV, supraspiracular; V, spiracular; VI, epipleural; VII, pleural; VIII, hypopleural or lateroventral; IX, adventral; X, ventral; and X^a, midventral.

PLATE 92

Neodiprion lecontei:

A.—Some defoliated twigs showing feeding on bark of stem.
B.—Eggs within needles of *Pinus virginiana.*


A


B

AMYLASE OF RHIZOPUS TRITICI, WITH A CONSIDERATION OF ITS SECRETION AND ACTION

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INTRODUCTION

That certain mold fungi secrete amyclastic and other enzyms has been known for a long time. However, much of the work in this direction has been centered around a few common forms, especially in the genera Aspergillus and Penicillium. In fact, the same organism has been selected by many investigators who studied the same or different phases of enzymic production. The literature on the subject is already very large and has been reviewed and listed in many of the publications of recent years. For this reason the writer will refer only to such articles in the body of the paper as are germane to the particular subject under discussion.

Rhizopus tritici was used for this investigation because it is responsible for large losses of sweet potatoes and other vegetables under storage and transportation conditions. Its parasitism has been proved repeatedly by inoculations into sweet potatoes, where it causes a rot identical in appearance with that produced by *R. nigricans*. Preliminary experiments were made with *R. nigricans*, which showed that it produces amylase in abundance. No attempt has been made to duplicate with *R. nigricans* the experiments carried out with *R. tritici*. So far as the writer is aware these are the first experiments of the kind conducted with *R. tritici*.

Some of the work of other investigators has been duplicated as far as the method employed would permit, the purpose being to compare *Rhizopus tritici* with some of the fungi hitherto studied. Some of the results of previous investigators were corroborated, while others were not, which indicates that no sweeping generalizations regarding all fungi can be drawn from the study of a single organism.

METHOD OF EXPERIMENTATION

The investigations were carried out mostly with the powdered mycelium, although the diffusion of the enzym into the culture solution was not entirely disregarded. For certain phases of the work extracts of the mycelium were used. The fungus was grown on a modified Czapek's nutrient solution or on sweet potato bouillon for most of the comparative

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studies. For some parts of the work Czapek's nutrient solution was preferable, since it was then possible to cultivate the fungus in a substrate of known composition. On the other hand, the fungus made a luxuriant growth on sweet potato bouillon, and for experiments, such as the influence of temperature on secretion, this medium was usually employed.

The fungus was grown in 2-liter Erlenmeyer flasks containing about 750 cc. of the sterile solution, on which enough fungous felt was produced to carry out several comparative experiments.

Preliminary experiments showed that the fungus grew poorly on a solution with sodium nitrate and cane sugar as a source of nitrogen and carbon, respectively. Ammonium nitrate was therefore substituted for sodium nitrate and glucose or potato starch, or both, for cane sugar in Czapek's nutrient solution. The composition of the solution as finally prepared is as follows:

Water	1,000.00 cc.
Magnesium sulphate (crystals)	.50 gm.
Potassium acid phosphate	1.00 gm.
Potassium chlorid	.50 gm.
Ferrous sulphate	.01 gm.
Ammonium nitrate	5.00 gm.
Glucose, starch paste, or both, in varying amounts to suit the requirements of the experiments as a source of carbon.	

The sweet potato bouillon is prepared as follows: To the peeled potatoes add double the weight of water; steam for one hour, then squeeze out the liquid through gauze; steam a second time, filter into flasks, and autoclave for 20 minutes at 13 pounds pressure. The sweet potato bouillon always contains a considerable quantity of reducing sugar and starch paste.

Rhizopus tritici grew well on both of these solutions and produced a thick, heavy felt in from 7 to 10 days at a temperature of 25° to 35° C. The better growth was made on the sweet potato bouillon. Contrary to what might be expected, starch paste was more efficient as a source of carbon in Czapek's modified nutrient solution than glucose. The organism was grown in incubators, the temperatures of which did not fluctuate more than 1°.

At the end of the growth period the mycelium, which formed a thick felt on the surface of the medium, was removed and washed in running water for about 15 minutes. It was treated subsequently according to Dox's (*9*) [1] modification of Albert and Buchner's "acetondauerhefe" method. After washing, the mycelium was stirred constantly in an excess of acetone for 10 minutes, squeezed as dry as possible, and treated a second time for 2 minutes in a fresh supply. This acetone was removed as in the former case, and the mycelium was treated with ether for 3

[1] Reference is made by number (italic) to "Literature cited," p. 784–786.

minutes. When air-dry the mycelium was put into small flasks and held at a temperature of 9° C. until required for use. Experiments to be discussed later will show that the mycelium can be held at 9° or even higher for several months without any appreciable loss in its ability to hydrolyze starch.

The hydrolysis by the mycelium or extract was carried out in 150-cc. pyrex flasks. A weighed portion of the mycelium was ground in fine quartz sand and transferred to the flasks, to which was added a measured quantity of the starch paste solution made in distilled water. While the percentage of starch is not material, a 0.5 per cent solution was used for most of the work. After the addition of 2 cc. of toluol to each flask as an antiseptic it was plugged by a cork with a small groove at the side to allow for the escape of the expanded air when steamed at the close of the experiment. Hydrolysis was carried out at different temperatures, the results of which are shown elsewhere.

C. P. chemicals were used in the preparation of the culture media. The Irish potato starch was obtained from Eimer and Amend. The sweet potato starch was prepared by the writer. Preliminary experiments showed that neither contained any reducing sugars. The sand used for grinding the mycelium was purified by washing in distilled water and then burning for an hour or more in a crucible. The water in which sand so prepared was suspended did not reduce copper.

At the close of the digestion period the enzym was inactivated by steaming the flasks in an Arnold steam sterilizer for about 15 minutes. To avoid evaporation during the process of heating, oiled paper was fastened with a rubber band over the cork and around the neck of the flask. Before this method was finally adopted tests were made to determine the temperature reached in a given volume of solution in a given length of time. Table I shows the results of these tests, made with tap water in Erlenmeyer flasks, with an initial temperature of 14° to 15° C. There was a small slit at the side of the cork to allow for expansion, and a thermometer was run through it, with the bulb submerged in the water.

TABLE I.—*Temperature reached by a certain volume of water when heated a given length of time in an Arnold sterilizer (average of several tests)*

Volume of water.	Capacity of flask.	Temperature.	Time.
Cc.	*Cc.*	*°C.*	*Minutes.*
50	100	80. 5	1
50	100	93. 0	2
100	100	69. 0	1
100	100	89. 5	2
100	100	96. 0	3
500	500	65. 0	2
500	500	79. 5	3

The loss of water by the use of the method described above was less than 0.1 gm. in a flask of 150-cc. capacity containing 100 cc. of solution.

After the flasks had been heated for 15 minutes the contents were filtered through a fine quality of absorbent cotton to remove the mycelium and sand. Filter paper was first tried but was finally rejected in favor of the cotton for two reasons: (1) The solution filtered slowly, thereby introducing considerable error as a result of evaporation; (2) it removed much of the nonhydrolyzed starch. After the filtrate cooled, the reducing sugars were determined volumetrically, according to the method of Clark (*8*). This is a quick and accurate method for the determination of small amounts of reducing sugars by titrating the reduced copper without removing it from the residual copper solution.

The results of starch hydrolysis set forth in the discussion of the following experimental data are expressed in milligrams of reducing sugars in a given volume of solution or in total reducing sugars formed. The results are expressed mostly in milligrams per 10 cc., because 10 cc. of solution are usually employed in making the titrations. If the quantity of reducing sugars in 10 cc. of solution is known, the total reduction or that portion of the starch remaining nonhydrolyzed can be calculated.

It is evident from the method employed that no account is taken of products intermediate between the starch and reducing sugars. It is likely that such products, for example dextrins, are formed in all cases, but the determination of the reducing sugar meets the requirements of the problem in hand, which has for its object mainly to show that a vigorous starch-splitting enzym is formed by *Rhizopus tritici*, and also some of the conditions upon which the production of this enzym depends and how certain environmental factors may influence its activity.

Various modifications of these methods were used in certain of the experiments, but such changes in the methods required to meet the needs of the experiments will be explained in sufficient detail when the results of the experiments are presented and discussed.

It was shown by Dox (*9*) that a considerable autolysis of the fungus mycelium actually takes place. In some enzym experiments where hydrolysis is measured by the amount of reducing sugars formed, a considerable error is likely to be introduced if a correction is not made for the autolysis of the mycelium itself. A number of tests have shown that the amount of autolysis produced from 0.25 gm. of mycelium suspended in 50 cc. of distilled water varies from 1.20 to 7.39 mgm. per 10 cc., with an average of 6.38 mgm. Where a considerable amount of reduction of the starch is involved, this amount would not introduce a very considerable error. On the other hand, where the total hydrolysis is small a considerable error in the final results might be introduced. In all experiments, except where the results would not be influenced one way or the other, the autolysis of the mold was determined and deducted from the total reducing sugars formed in the system.

EXPERIMENTAL DATA

HYDROLYSIS OF RAW STARCH

Preliminary experiments showed that *Rhizopus tritici* produced an enzym which hydrolyzed starch to reducing sugars. This fungus is commonly found as a cause of the decay of sweet potatoes in storage and along with *R. nigricans* probably is responsible for the greater percentage of decay attributed to the Mucoraceæ. In just what form they utilize carbohydrates when growing on the sweet potato is not known, but that they are responsible for certain carbohydrate changes in the host directly through their own activity or by stimulating the host to do so, or both, will be shown by investigations now under way.

Most of the previous work with amylase secreted by fungi was carried out with starch paste or soluble starch. This obviously is not the form in which it occurs in the host, and although the enzym might digest starch paste, it is not safe to conclude that it would act on raw starch, or if at all, to the same degree.

Ward (*24*) concluded from the appearances of the starch grains of the Irish potato that they were not acted on by Pythium, while Hawkins and Harvey (*14*), on the other hand, found from a chemical determination of the total starch present in the sound and rotted portions of the same potato that the starch content was actually lower in the latter than in the former. That all fungi do not behave the same as regards their action on starch is evident from the fact that Hawkins (*13*) found that neither *Fusarium oxysporum* nor *F. radicicola* apparently alters the starch content of Irish potato. It is evident from the results of the authors just cited that no general conclusions can be drawn for all fungi from the behavior of any one or more fungi. The first experiments, therefore, were designed to test the comparative hydrolysis of raw starch and starch paste. The results are given in Table II.

TABLE II.—*Results of hydrolysis of raw starch expressed in terms of reducing sugar (average of several tests)*

Mycelium.	Water.	Starch.	Time of hydrolysis.	Temperature.	Hydrolysis in milligrams per 10 cc. of solution.	Total hydrolysis.	Source of starch.
Gm.	*Cc.*	*Per cent.*	*Hours.*	*° C.*		*Mgm.*	
0.10.....	50	0.5	5.0	27.5	1.06	5.30	Sweet potato.
.20.....	100	.5	18.0	27.5	5.945	59.45	Do.
.20.....	100	.5	17.5	27.5	7.38	73.80	Do.
.20.....	100	.5	17.5	27.5	7.07	70.70	Do.[a]
.20.....	100	.5	17.5	27.5	6.28	62.80	Irish potato.
.20.....	100	.5	17.5	27.5	9.12	91.20	Do.[a]

[a] Starch macerated in sand before hydrolysis was started.

While an examination of Table II shows that both raw Irish and sweet potato starch are hydrolyzed, no large amount of invert sugars are produced after hydrolyzation is carried on for 17.5 to 18 hours. Grinding the starch in fine quartz sand does not seem to influence the amount of hydrolysis appreciably.

That starch paste is more readily hydrolyzed than raw starch is evident from the results of the following experiments. Two sets of flasks were prepared to contain 0.2 gm. of powdered mycelium. To one set were added 100 cc. of sterile distilled water and 0.5 gm. of raw sweet potato starch, and to the other 100 cc. of water containing 0.5 gm. of starch paste. A third set contained 100 cc. of water and 0.2 gm. of mycelium but no starch. Toluol was added as an antiseptic. Hydrolysis was carried on for 18 hours at 40° C. In the set with water and mycelium 1.98 mgm. of reducing sugar, representing autolysis of the fungus, were found per 10 cc. of solution. This amount of reducing sugar was deducted from the results obtained from the other two sets. Reducing sugars equivalent to an average of 1.20 mgm. per 10 cc. of solution were obtained from the raw starch, while 27.95 mgm. were obtained per 10 cc. from the starch paste solution, or an amount more than 23 times as large.

INFLUENCE OF AGE OF MYCELIUM ON POWER OF HYDROLYSIS

To carry out any considerable number of comparative experiments at different times the mycelium must be produced in quantity and kept for some time. Before such material could be used for comparative studies it was necessary to determine whether the mycelium lost its power of digestion with age, and if so to what extent.

The mycelium was grown in large flasks on sweet potato bouillon. At the end of 8 days' growth it was removed and prepared according to the method already described. Hydrolysis was carried out at different times at a temperature of 27.5° C. for 19 hours by the use of 0.25 gm. of powdered mycelium. A starch paste solution was prepared which contained 53.4 mgm. of starch per 10 cc. of solution. This sterilized starch solution was tightly stoppered to prevent evaporation and contamination and was stored at a temperature of 9°. Two days after the mycelium was collected the first experiment was conducted. Fifty cc. of the starch paste and 0.25 gm. of the mycelium finely ground in sand were used in 150-cc. pyrex flasks, with 2 cc. toluol added as an antiseptic. Two flasks with mycelium and starch paste and one control flask containing mycelium and 50 cc. of water were used in each test. The amount of autolysis was deducted from the average of two closely agreeing samples. The results appear in Table III.

There was a slight decrease in the amount of reducing sugars in the tests of the last three months. From the results it seems safe to conclude that the mycelium may be kept for several months without any appreciable

loss in reducing power. These results are in accord with those of Dox (*9*), who found that mycelium may be kept almost indefinitely without losing its activity.

TABLE III.—*Amount of reducing sugars produced by the same samples of mycelium used at different times*

Feb. 20.	Mar. 5.	Mar. 18.	May 7.	June 10.	June 25.	Sept. 26.
Mgm. 216. 337	*Mgm.* 225. 5	*Mgm.* 222. 65	*Mgm.* 233. 1	*Mgm.* 208. 55	*Mgm.* 205. 4	*Mgm.* 204. 9

INFLUENCE OF DIFFERENT TEMPERATURES ON THE AMYLOCLASTIC ACTIVITY OF THE MYCELIUM

Although it was shown by Table III that mycelium may be stored at 27.5° C. for a number of months without materially affecting the activity of the enzym, it can not be concluded that it can be kept unimpaired at any temperature. As a matter of fact, the following results show that the hydrolytic power of the enzym is somewhat impaired when held for a time at a high temperature.

The mycelium for these experiments was produced in six 2-liter flasks containing about 750 cc. of sweet potato bouillon. At the end of the growth period the mycelium was made into one composite sample and held at a temperature of 9° C. for 18 hours. A sample was then removed and its original hydrolytic power was determined. The remainder was divided into three lots, one being stored at 9°, one at 35°, and one at 60°.

To determine the original hydrolytic power of the mycelium two 0.25-gm. lots were weighed out and ground in fine quartz sand. To one flask containing enzym powder were added 100 cc. of a 0.5 per cent starch paste solution and to the other 100 cc. of sterile distilled water. After the addition of toluol as an antiseptic both were digested for 18 hours at 40° C. In the former 2.2 mgm. and in the latter 33.46 mgm. of reducing sugars were found in 10 cc. of solution, or a total of 22 mgm. and 334.6 mgm. in 100 cc., respectively. These figures will serve as a basis for comparison of future tests of the same lot of mycelium stored at different temperatures. (Table IV.)

TABLE IV.—*Amount of starch hydrolized by mycelium stored at different temperatures for a given length of time*

[Expressed in milligrams per 10 cc. of solution]

Temperature.	Original sample before storage.	After 12 days' storage.	After 39 days' storage.	After 73 days' storage.
°C.				
9	33. 46	29. 649	39. 429	37. 448
35	.....	35. 148	37. 740	30. 100
60	.....	22. 428	21. 400	17. 000

The results show that the hydrolytic power of the mycelium stored at 60° C. at the end of 73 days is somewhat more impaired than that of mycelium stored at 35° and 9° for the same length of time. On the other hand, the results indicate that the mycelium may be safely stored for a considerable time at 9° and 35° without materially affecting the enzym.

EFFECT OF TEMPERATURE ON THE HYDROLYTIC POWER OF THE ENZYM

It is generally understood that enzyms are more resistant to heat when in the form of a powder than when in suspension. Kjeldahl (*18*) found that the action of amylase at 0° C. was very slow but increased rapidly with the increase in temperature up to 60° and at 70° became insignificant. Similar results were obtained by Durandard (*11*), who reports that the optimum temperature for the hydrolysis of rice starch by an extract of *Rhizopus nigricans* to be 45°. He obtained some hydrolysis at 10° and four times as much at 45° as at 30°. It diminishes rapidly toward 55°, becoming very feeble at 60° and nothing at 70°. The writer found likewise the optimum temperature for the hydrolysis of potato starch to be about 45°, with a gradual decrease above that temperature, becoming practically nothing at 60°. Effront (*12*) concludes also that the temperature has no other effect than to reduce the diastatic power, and the nearer the temperature approaches 70° the greater is the reduction. White (*26*) found that certain enzyms in dry oats, among them diastase, were not injured on heating for 4½ hours to 100°, but that an exposure for one hour at 130° did destroy the ferments.

That the amylase contained in *Rhizopus tritici* is destroyed at a temperature of 60° C. is shown in the following experiments. Five-tenths gm. of mycelium was extracted for 24 hours in each of two flasks containing 150 cc. of sterile distilled water at a temperature of 9°. The contents of the flasks were then filtered, and 100 cc. were pipetted into 250-cc. flasks. Both flasks were exposed for an additional 100 hours, one at a temperature of 60° and one at 9°. The contents of each flask were then diluted with 100 cc. of a 1 per cent starch paste and hydrolyzed for 18 hours more at 40°. At 60° and 9° the reducing sugars formed per 10 cc. of solution were on an average 1.36 and 36.36 mgm., respectively. Although a little reducing sugar was formed, it is believed that it was derived by autolysis of the mycelium during the period of extraction.

INFLUENCE OF GLUCOSE ON THE HYDROLYSIS OF STARCH

The stimulating and retarding effect of certain substances, especially those identical with or similar to the products of hydrolysis, have been subjects of investigations for a long time. Hill (*15*) found that glucose interfered with the action of maltose, and Armstrong (*1*) pointed out a number of cases where the reaction products inhibited the action of the

enzyms. Kellerman (*17*) found that the alkalies without exception seemed to be detrimental and the metals generally injurious to the action of Taka diastase. From the results obtained by these and other investigations it is evident that many substances influence the rate of action of the enzym. The data shown here are the results of a single experiment. Four flasks marked a, b, c, d were prepared, each to contain 0.25 gm. of powdered mycelium. A second lot of flasks was prepared, and into flask a were added 100 cc. of a 0.5 per cent starch paste solution; into flask b 125 cc. of a 0.5 per cent starch paste and 0.625 gm. glucose; into flask c 125 cc. of a 0.5 per cent starch paste and 2.5 gm. glucose; into flask d 125 cc. of a 0.5 per cent starch paste and 6.25 gm. glucose. After thorough mixing, 25 cc. were drawn from flasks b, c, and d, and the reducing sugars were determined volumetrically. The contents of flasks b, c, and d were then poured into the corresponding flasks containing mycelium and digested for 18 hours at 40° C., with the results given in Table V.

TABLE V.—*Amount of reducing sugars before and after hydrolysis*

[Expressed in milligrams per 10 cc. of solution]

Sample.	Reducing sugars originally present.	Reducing sugars present at end of the digestion period.	Increase in reducing sugars.
a	0	42. 476	42. 476
b	53. 040	86. 899	33. 859
c	181. 580	216. 080	34. 500
d	438. 386	472. 108	33. 722

It seems evident from the results of a single test that the presence of glucose decreases the activity of the amylase, since the total reducing sugars formed in sample a is considerably greater than in samples b, c, and d. On the other hand, the closely agreeing results of b, c, and d indicate that the amount of glucose present at the strength used in this experiment has no effect upon the hydrolysis of the starch.

RELATION OF QUANTITY OF STARCH PRESENT TO AMOUNT OF HYDROLYSIS

This subject naturally involves a consideration of the law of "mass action," and in the literature on this subject there appears to be no agreement of opinion on the question. The investigations show that so far as enzyms are concerned so many factors influence the reaction that no definite conclusion can be drawn. For example, Brown and Glendinning (*4*) showed that when the concentration of the enzym relative to the starch in the early stages is very small, the amount of starch hydrolyzed per unit volume will be very large compared with the amount of the combination of starch and enzym. If the concentration of the unchanged substrate remains very large in relation to that of the

combination, the latter will remain nearly constant in amount and equal amounts of starch will be hydrolyzed in equal times, the curve being a straight line. On the other hand, when the concentration of the starch has been greatly reduced, the amount of the combination and accordingly the hydrolysis will follow more closely the law of "mass action." Similar results were obtained by Armstrong working with lactose, maltose, and emulsin. Other investigators have found various factors influencing the reaction between the enzym concerned and the substrate. For a full consideration of the theory involved in "mass action" the reader is referred to a discussion of the subject by Bayliss (*2*).

The data submitted in Table VI are the results of a considerable number of experiments which were varied to suit the requirements of the problem. In the first series of experiments the amount of enzym power (0.25 gm.) was constant and the volume of the starch paste solution was varied. The time of hydrolysis was 19 hours at 32° C.

TABLE VI.—*Total amount of reducing sugars and reducing sugars per 10 cc. of solution in different volumes of a 0.5 per cent starch paste solution*

Sample.	Volume of solution.	Reducing sugars per 10 cc.	Total reducing sugars.
	Cc.	*Mgm.*	*Mgm.*
a	50	39.984	199.92
b	100	25.864	258.64
c	150	26.600	399.00
d	200	16.400	328.00

In sample a the reducing sugars per 10 cc. is considerably larger than in sample d, while b and c are about the same. In total reducing sugars found there is a progressive increase up to and including 150 cc., and then a slight decrease. While in sample a some starch yet remained nonhydrolyzed, it is likely that on approaching the end point the rate of hydrolysis was slowed up. It is probable that a shorter period of hydrolysis would have given a different curve and that the total reducing sugars formed would have paralleled the reducing sugars per 10 cc.

Somewhat similar results were obtained when the total volume of solution (100 cc.) and the amount of enzym powder (0.25 gm.) were constant but the quantity of starch paste was varied. A 1.5 per cent starch paste solution was used in the dilutions, enough distilled water being added to make a total volume of 100 cc.

The time of hydrolysis was 19 hours at 32° C. The average results of parallel tests are shown in Table VII.

The results show an increase in reducing sugars with the increase in the amount of starch present from sample a to sample c, inclusive, and then a slight decrease. In sample a, although the end point had been more closely approached than in any of the other samples, some starch still remained unhydrolyzed. If it were not for the results obtained in samples d

and e, it might be assumed that the accumulation of reducing sugars acted as a paralyzer to further action of the enzym or, as has been suggested by some investigators, the enzym entered into combination with the products of the hydrolysis and consequently became inactive.

TABLE VII.—*Amount of 1.5 per cent starch paste used, total reducing sugars, and reducing sugars per 10 cc.*

Sample.	Total volume of solution.	Volume of starch paste.	Reducing sugars per 10 cc.	Total reducing sugars.
	Cc.	*Cc.*	*Mgm.*	*Mgm.*
a	100	20	21. 240	21. 24
b	100	40	35. 632	356. 32
c	100	60	37. 842	378. 42
d	100	80	33. 498	334. 98
e	100	100	32. 551	325. 51
f	100	00	5. 198	51. 98

In the series of experiments reported in Table VIII different amounts of a 1 per cent starch paste solution were used, and enough water was added to make a total volume of 500 cc. One-fourth gm. of enzym powder was added to each set of flasks. The time of hydrolysis was 18 hours at 40° C.

TABLE VIII.—*Amount of 1 per cent starch paste used, total reducing sugars, and reducing sugars per 10 cc.*

Sample.	Total volume of solution.	Volume of starch paste.	Reducing sugars per 10 cc. of solution.	Total reducing sugars.
	Cc.	*Cc.*	*Mgm.*	*Mgm.*
a	500	20	3. 1850	159. 250
b	500	50	6. 3050	315. 250
c	500	100	8. 9375	446. 875
d	500	200	10. 4000	520. 000
e	500	300	11. 9600	598. 000
f	500	400	12. 1550	607. 750
g	500	500	8. 1575	407. 875

The amount of reducing sugars per 10 cc. increases with the increase in the amount of starch from sample a to sample f and then decreases. An approach toward the end point might here also account for the lesser amount of hydrolysis in the more dilute solutions if the total reduction in sample g, which contains the largest amount of starch, was not actually less than in several of the other samples.

A final series of experiments was carried out in which the total volume of 0.5 per cent starch paste was varied but the amount of enzym powder (0.25 gm.) was constant. Hydrolysis was carried on for 18 hours at 40° C. (Table IX.)

There was a decrease in the reducing sugars per 10 cc. and an increase in total sugars as the volume of the solution increased from sample a to sample e, and then a reverse of the process.

TABLE IX.—*Volume of 0.5 per cent starch paste solution used, total reducing sugars, and reducing sugars per 10 cc.*

Sample.	Volume of starch paste.	Reducing sugars per 10 cc.	Total reducing sugars.
	Cc.	*Mgm.*	*Mgm.*
a	50	45. 16	225. 8
b	100	37. 64	376. 4
c	200	28. 28	565. 6
d	300	19. 74	592. 2
e	400	14. 42	576. 8
f	500	9. 80	490. 0

IS AN END POINT IN HYDROLYSIS REACHED?

Theoretically an end point should not be reached without shifting the point of equilibrium of the solution. As a matter of fact, to settle the question is difficult by any method, since there may be intermediate products between starch and reducing sugars which are not revealed by the iodin test and do not reduce copper. The experiments were made with extracts of the mycelium. The mycelium (1.5 gm.) after powdering was extracted in a pyrex flask for 24 hours at 9° C. in 300 cc. of distilled water. The extract was then filtered. Two hundred fifty cc. of the extract were then diluted with 250 cc. of a 2 per cent starch paste solution. After thorough mixing 20 cc. were drawn off, 2.5 cc. concentrated hydrochloric acid were added and the mixture was hydrolyzed by boiling for 2.5 hours. The solution was neutralized with sodium hydroxid made up to 200 cc. with water, and the starch present was determined as reducing sugars. A preliminary test showed that no reducing sugars were present in the original starch paste solution. After hydrolysis reducing sugars equivalent to 104 mgm. of starch per 10 cc. were found.

The solutions were mixed on May 22 and hydrolysis carried out at 45° C. Reducing sugars were determined approximately 24 hours apart for several days thereafter with the results shown in the Table X.

TABLE X.—*Amount of reducing sugars at different dates and equivalent in starch*

[Expressed in milligrams per 10 cc.]

Date.	Reducing sugars.	Equivalent in starch.
May 23	56. 516	52. 560
24, 9.30 a. m	79. 236	73. 689
24, 3.30 p. m	84. 518	78. 602
26	99. 968	92. 970
27	103. 092	95. 875
28	105. 364	97. 988
29	108. 866	101. 245
30	108. 866	101. 245
31	108. 866	101. 245
June 9	108. 889	101. 267

The results show that the amount of reducing sugars steadily increased for the first 7 days but remained practically stationary thereafter. At the end of 18 days a small amount of starch yet remained nonhydrolyzed.

To determine whether the addition of a small amount of starch would stimulate further hydrolysis, 100 cc. of the solution described on page 772 were mixed with 100 cc. of an approximately 0.5 per cent starch paste solution. A small amount (20 cc.) was drawn off, and the actual amount of starch was determined. The remainder was hydrolyzed at 45° C.

After acid hydrolysis reducing sugars to the amount of 1,568 mgm. were found in 200 cc. of the original solution. Of this amount 1,088.89 mgm. of reducing sugars and 27.33 mgm. of nonhydrolyzed starch (equivalent to 29.38 mgm. reducing sugars) were brought over to the solution when the dilution was made, making a total of 1,118.27 mgm. reducing sugars. Deducting this amount from the amount originally found (1,568 less 1,118.27 mgm.), the result gives the amount of reducing sugars added in the form of starch, or 449.73 mgm. This is calculated to be equivalent to 418.2489 mgm. of starch. To this amount should be added 27.33 mgm., the quantity of nonhydrolyzed starch present before the solutions were mixed, making a total of 445.58 mgm. starch present in 200 cc. of the solution when hydrolysis was started. After hydrolysis had gone on for 24 hours a sample was taken, and the reducing sugars were determined, which gave in 200 cc. a total of 1,516.6 mgm. There was no starch left in the solution according to the iodin test. Since in the original solution there were 1,568 mgm. of reducing sugars present, 51.4 mgm. (equivalent to 47.8 mgm. starch) remain unaccounted for, except as intermediate products between starch and reducing sugars.

Parallel experiments, which will not be given in detail, gave similar results.

The evidence brought out shows that an equilibrium is established in the solution before quite all the starch is hydrolyzed. Also that if more starch is added and the solution is diluted the starch finally disappears so far as its presence is indicated by the iodin test.

So far as these and many other results go, an end point is reached if the disappearance of the starch alone is considered. Viewed from the standpoint of reducing sugars found, an end point is not reached. Many experiments not designed primarily to demonstrate this point have shown that no starch, as indicated by iodin, remains in the solution after a definite length of time. On the other hand, starch is shown to be present in some solutions by the same test after a considerable time. It was also shown by experiments that if an end point was not reached at a certain temperature, namely 45° C., the starch would completely disappear in 24 hours by shifting the solution to a temperature of 35°.

Perhaps an explanation of some of these facts may be found in the results of other investigations. The results of the above experiments show that all the starch was not accounted for as reducing sugars,

although in such solutions no starch was present, if judged by the iodin test. This difference might be explained by the presence of dextrins as intermediate products. Brown (*3*) claims that in the action of diastase on starch the reaction ends when the composition of the product is 80.8 per cent maltose and 19.2 per cent dextrin. Maquenne and Roux (*21*), however, suggest that the equilibrium of 80.8 per cent maltose and 19.2 per cent dextrin referred to above is due to insufficient activity of the enzym and that if malt diastase is activated by acid in small amount the whole of the starch is found to be converted into sugar, so no dextrins remain. Bayliss (*2*) found that the amount of maltose produced in the first stage was greater than the equilibrium position of Brown and Heron because it was allowed to proceed for a longer time.

Although the writer did not use a temperature above 40° C., this temperature might have had some bearing on the proportion of sugar to dextrins, in accordance with the interesting results of Brown and Heron (*5*).

These investigators found that the dextrinase is more injured by a temperature of 68° C. than the amylase. According to this theory they explain the fact that when starch paste is acted on by diastase which has been exposed to a temperature of 68° there is less maltose and more dextrin formed than when the enzym has not been so heated. This raises the question as to just where the influence of temperature makes itself felt. Furthermore, facts which might bear upon the question were brought out by Tammann (*23*), who reports that an increase of hydrolysis was obtained in a stationary system by altering any of the other conditions of the equilibrium, such as the addition of more amygdalin, renewal of the products of the reaction, raising the temperature, or increasing the dilution. In Tammann's work the retardation would virtually be due to the accumulation of the products of the reaction.

GROWTH AND HYDROLYSIS IN A SOLUTION OF STARCH PASTE

The remarkable power of *Rhizopus tritici* to grow on almost any kind of medium is evident when we consider that it can be isolated from a great variety of decayed substances. Its ability to hydrolyze starch in a solution poor in nutrient material was tested several times by inoculating a starch paste solution made with distilled water. While such a solution would contain nutrient substances in addition to the carbohydrates introduced in the form of starch, a considerable growth would hardly be expected, but, nevertheless, a fair growth was made and hydrolysis of the starch went on.

The experiments were made in Erlenmeyer flasks containing 500 cc. of a 0.5 per cent starch paste solution. Some of the inoculations were made with bits of mycelium and spores and some with spores alone. Growth was slow at the outset, the colonies being submerged

at first, a felt forming later on the surface of the liquid. The solutions were tested for reducing sugars at the beginning of the experiments, but in no case were any found. The fungus must then of necessity have either to utilize the starch directly or first have converted it into some simpler form. From time to time some of the liquid was drawn off, and the reducing sugars were determined. The results showed an increasing amount of reducing sugars present with each subsequent determination, from which it is evident that the fungus hydrolyzed the starch in excess of its needs. If the growth continued long enough the solution which was milky in color at first finally became clear, showing that practically all the starch was hydrolyzed. Many experiments in the course of these investigations likewise demonstrated clearly that the fungus hydrolyzed the starch in the solution, although reducing sugars were already present. Furthermore, the hydrolysis of the starch in a solution of starch and glucose began very soon after inoculation, which suggests that the enzym diffuses into the solution soon after the beginning of growth. This subject will receive further consideration in the discussion of an extracellular enzym.

EXTRACELLULAR ENZYM

The results in the following experiments show other interesting facts in addition to the production of an extracellular amylase. Two nutrient solutions a and b, differing in the source of nitrogen, were used. Solution a had the following composition:

Water	1,000.00 cc.
Magnesium sulphate (crystallized)	.50 gm.
Potassium acid phosphate	1.00 gm.
Potassium chlorid	.50 gm.
Ferrous sulphate	.01 gm.
Sodium nitrate	2.00 gm.
Starch	10.00 gm.

Solution b differed from a in that the sodium nitrate was replaced by 5 gm. of ammonium nitrate.

The chemicals were first dissolved in the water by steaming, after which the starch was added and the entire mixture was sterilized by autoclaving.

The growth in these two solutions was remarkably different. In a the mycelium was mostly submerged, while in b a thick felt was formed on the surface. Solution a produced in 16 days of growth a total dry weight of 0.0298 gm.; b, 0.7198 gm., or about 24 times as much. Both solutions were inoculated on October 27. The reducing sugars and starches were determined at stated intervals thereafter, as shown in Table XI.

TABLE XI.—*Amount of reducing sugars and starch present in solutions a and b at stated intervals of time*

[Expressed in milligrams per 10 cc. of solution]

Date.	Solution a.		Solution b.	
	Reducing sugars.	Starch.	Reducing sugars.	Starch.
Oct. 27	0	111.0	0	106.0
29	5.3	103.0	5.8	96.0
31	21.7	83.0	34.6	47.0
Nov. 3	46.0	59.0	35.2	24.0
5	61.4	41.0	21.6	25.0
7	65.3	38.5	14.0	23.8
10	75.0	25.2	7.7	22.8
12	75.8	23.4	6.0	21.6
3 (controls)	0	.....	0	.....
12 (controls)	0	.....	0	.....

From Table XI it is seen that in two days reducing sugars in excess of those used by the fungus were produced with a decrease in the amount of starch. In the a solution the reducing sugars gradually accumulated to the end of the experiment, while the amount of starch decreased, showing that the fungus did not use a corresponding amount of the reducing sugars formed. On the other hand, in solution b the reducing sugars increased up to November 3 and then decreased to the close of the experiment, while the starch decreased rapidly to November 3 and very little thereafter, which suggests that hydrolysis was slowed up as it approached the end point and did not keep pace with the demands of the fungus for reducing sugars. This condition is reflected in the amount of dry matter formed, which is about twenty-four times greater in solution b than in solution a. The amount of starch in the two solutions at the close of the experiment was practically the same. It seems, then, that an extracellular amylase was promptly secreted by the fungus and that it hydrolyzed the starch in excess of the needs of the fungus in one case (a) to the close of the experiment and in the other until November 3, when the reducing sugars consumed exceeded those produced by the hydrolysis of the starch.

Why the difference in the composition of the two solutions plays such a fundamental rôle in the growth of the fungus can not be answered. As previously stated, solution a derives its nitrogen from sodium nitrate and solution b from ammonium nitrate. The growth in the latter case was many times greater than in the former. Since solution a was virtually Czapek's nutrient solution, it was tried at the outset for other work of a similar nature and was later modified by the substitution of ammonium nitrate for sodium nitrate. The solution so modified gave a luxuriant growth of mycelium. Solution a, however, apparently had no inhibitory action on the amylase, so that hydrolysis of the starch went on unhindered.

REMOVAL OF AMYLASE BY FILTERING

The enzym powder was extracted for 24 hours in sterile distilled water. The contents of one set of flasks was filtered through absorbent cotton, which removed the fragments of mycelium, and the others were filtered through four thicknesses of No. 1 Whatman chemically prepared filter paper. A quantity of this filtered extract was then mixed with an equal volume of a 1 per cent starch paste solution and hydrolyzed for 18 hours at 40° C. At the close of the period of hydrolysis the reducing sugars were determined in the usual way. The average of several parallel experiments showed that when filtered through cotton, 172.51 mgm. reducing sugars were formed in 100 cc. of solution but that only 129.32 mgm. were formed when filtered through filter paper.

INFLUENCE OF TEMPERATURE AT WHICH MYCELIUM IS GROWN ON ITS POWER OF HYDROLYSIS

The investigations of the writer and others have shown that the optimum temperature for the activity of amylase is about 45° C. and that activity is reduced by higher and lower temperatures. Since these results, however, were obtained from mycelium grown at one temperature, the question was naturally suggested whether the temperature at which it was grown did not influence the amount of amylase produced. The mycelium was grown on sweet potato bouillon in 2-liter Erlenmeyer flasks. One set of flasks was incubated at 9°, one at 29°, and one at 40°. At the close of the incubation period (10 days) the mycelium was removed from the flasks and treated with acetone and ether in the usual way. The mycelium from the flasks held at the same temperature was made into a compound sample and stored at 9° until used.

The hydrolytic power of the enzym was determined by the use of 0.25 gm. of powder in all tests but two. With the smaller amount of enzym powder hydrolysis was carried out with 50 cc. of a 0.5 per cent starch paste solution; with all others 100 cc. were used. The time of hydrolysis was 18 hours at 40°. At the close of the experiment, the enzym was inactivated by steaming for 10 minutes. The results are given in Table XII.

TABLE XII.—*Results of hydrolysis of starch by mycelium grown at different temperatures*

Temperature.	Milligrams reducing sugars per 10 cc.
°C.	
9	39.700
29	26.854
40	9.933

The results show a very striking influence of the temperature on the production of amylase. A temperature of 40° C. represents about the maximum temperature for growth and 9° the minimum, while a good

growth occurs at 29°. At first thought one might suspect that at the higher temperature the enzym diffuses out into the solution more readily than at the two lower temperatures, and, indeed, one can not say such is not the case. If the hydrolytic capacity of the enzym corresponded to the growth of the fungus in the nutrient solution, as it does not, such a theory might receive strong support. The poorest growth is at the lowest temperature. At 9° the mycelium was mostly submerged, and no fruiting had taken place. On the other hand, at 29° and 40° a thick felt had formed, with some fruiting, though less at 40° than at 29°.

QUANTITATIVE REGULATION OF AMYLASE

The results of many investigations have shown a quantitative regulation of certain enzyms of various fungi. Brunton and MacFayden (7) found that a bacterium produced diastase when cultivated on starch paste but not when grown on meat broth. In the latter case a peptonizing enzym was produced. Pfeffer (*22*) found that in several mold fungi the secretion of diastase depended upon similar conditions, and Brown and Morris (*6*) claim a similar regulatory action with barley, in that when readily assimilable substances were supplied the secretion of diastase did not take place, but when no such substances were available diastase was formed at once. It was likewise found by Wortmann (*27*) that certain molds had the power of excreting a starch-dissolving enzym when starch grains were the only available food and that no secretion took place if sugar or tartaric acid was offered to the organism along with the starch. More recent workers have arrived at similar results with different fungi. Went (*25*) showed that *Monilia sitophila* secreted a number of enzyms, some of which were produced only when the particular substance on which they act was present in the culture solution. Others were produced when substances chemically allied to the products of hydrolysis were present. In general, however, he concluded that the secretion of enzyms was not a hunger phenomenon, since those fungi which were best nourished produced the most enzym. Dox (*9*), on the other hand, demonstrated that for *Penicillium camemberti*, at least, the enzyms were secreted regardless of the chemical nature of the substrate. He found that by cultivating the fungus on a particular substratum the quantity of the corresponding enzym may be increased, but that no enzym not normally produced by the organism could be developed by any special method of nutrition. Katz (*16*) in 1898 published the results of the regulating action of certain chemical substances in the solution of the regulatory secretion of amylase by *P. glaucum*, *Aspergillus niger*, and *Bacillus megatherium* and found that while the amylase secretion was not prohibited by the presence of substances chemically allied to starch, their effect was greatly to inhibit it. He found that the different fungi did not respond exactly in the same way and cites as proof the results with *A. niger* and *P. glaucum*. The presence of sugars in the

solution had a much less inhibitory effect on the production of amylase with *A. niger* than with *P. glaucum*. Similar conclusions were reached by Duclaux (*10*) with *P. glaucum* and *A. glaucus*, though he considered only the enzyms which diffused into the culture medium. The investigations of Kylin (*20*) with *P. glaucum*, *P. biforme*, and *A. niger* corroborate in a general way the results of other investigators. He found no qualitative regulation of the enzyms studied by him (diastase, invertase, and maltase), though a quantitative regulation was conclusively proved. With *P. glaucum* the regulating secretion of diastase was greater than with *A. niger*. Knudson (*19*), on the other hand, demonstrated a qualitative regulation of tannase with *A. niger* and *P.* sp. These fungi produced gallic acid by the fermentation of tannic acid when the latter was added to a modified Czapek's nutrient solution, but if supplemented with sucrose no tannase was formed. A number of other substances as a source of carbon likewise failed to stimulate the secretion of tannase. Young (*28*) studied the inulase formation by *A. niger* in a nutrient solution and found a well-marked quantitative regulation of the production of the enzym. He showed that inulase was produced in greatest amount in the mycelium (extracellular enzyms not studied) when inulin was used as the source of carbon but was likewise produced when other carbohydrates were employed. The substances most closely allied to inulin were most efficient in the production of the enzym.

The results of the writer's experiments which follow demonstrate also a quantitative regulation of amylase in nutrient solutions. Sweet potato bouillon and Czapek's modified nutrient solution (see p. 762) with glucose and starch in combination or alone in varying amounts were used as substrates.

In all these experiments the fungus was grown in 2-liter flasks containing 1,000 cc. of solution. At the end of the growth period the mycelium was removed and prepared in the usual way, according to the "acetondauerhefe" method of Albert and Buchner, the mycelium from the flasks of each series being mixed together to make a compound sample.

EXPERIMENT 1.—The fungus was grown on Czapek's modified nutrient solution with glucose or starch or both as a source of carbon. The cultures were incubated for 8 days at 32° C. Hydrolysis of starch was carried out for 19 hours at 32° by using 0.25 gm. of enzym powder in 50 cc. of a 0.5 per cent starch paste solution. (Table XIII.)

TABLE XIII.—*Source of carbon in Czapek's modified nutrient solution and amount of hydrolysis by the enzym powder per 10 cc. of the substrate*

Series.	Starch.	Glucose.	Reducing sugars.
	Gm.	*Gm.*	*Mgm.*
a	5	5	26. 19
b	0	5	34. 10
c	5	0	39. 28

EXPERIMENT 2.—In this set of experiments sweet potato bouillon was compared with Czapek's modified nutrient solution, the latter containing different amounts of starch and glucose as a source of carbon. The reducing sugars were determined in each series before inoculation and after the fungous growth had been removed, the enzyms in the solutions being inactivated at the end of the growth period by autoclaving the solutions. The cultures were incubated for 10 days at 35° C. The hydrolytic power of the enzym was determined by the use of 0.25 gm. of powder in 100 cc. of a 0.5 per cent starch paste solution. The time of hydrolysis was 18 hours at 40° C. (Table XIV.)

TABLE XIV.—*Source of carbon in Czapek's modified nutrient solution, amount of reducing sugars before and after the growth of the fungus, and the hydrolysis by the enzym powder*

[Expressed in milligrams per 10 cc. of the substrate]

Series.	Starch.	Reducing sugars.		Hydrolysis by enzym powder.
		Before inoculation.	After removal of fungous growth.	
	Gm.			
a	5	0	6.599	13.21
b	0	112.186	40.900	5.16
c	5	112.830	16.882	6.05
d	Not determined [a]	220.570	92.863	24.14

[a] Solutions a, b, and c were Czapek's nutrient solution; d was sweet potato bouillon. The reducing sugar in b and c before inoculation was glucose.

The starch was not determined, but it was shown to be present in series a, c, and d by iodin before the solutions were inoculated. When the fungous growth was removed the starch had all disappeared in series a and d.

From the results it is seen that the largest amount of hydrolysis took place with mycelium grown on sweet potato bouillon (d), where reducing sugars and starch both were originally present. On the other hand there was considerably more hydrolysis with mycelium grown on starch alone as a source of carbon (a) than where glucose was used alone (b) or in combination with starch (c).

The reducing sugars in series b, c, and d were considerably less at the end of the growth period than at the outset, showing that the fungus made use of reducing sugars or had converted them into other substances,possibly alcohol, acids, etc. No starch remained in the solutions. In series a the starch had entirely disappeared, but a small amount of reducing sugar was present. In this case also the fungus had either used a considerable amount of carbohydrate or had converted it into other compounds.

The fungus made the best growth in series d, but it was good in all and fruited abundantly in each of the solutions.

EXPERIMENT 3.—In the following experiments, Czapeck's nutrient solution was used for series a, b, and c, and sweet potato bouillon was used for d, the reducing sugars (glucose in b and c) and starch being determined in the solutions before and after the growth of the fungus. The digestion period was 12 days at 35° C.

In these experiments no account is taken of the amount used by the fungus or that converted to other compounds by it.

The digestive power of the mycelium was determined by using 0.2 gm. enzym powder in 100 cc. of a 0.5 per cent starch paste solution, which was hydrolyzed for 18 hours at 40° C. (Table XV.)

TABLE XV.—*Amount of reducing sugars and starch in solutions before and after the growth of the fungus; also the hydrolysis of starch by enzym powder*

[Expressed in milligrams per 10 cc. of solution]

Series.	Before inoculation.			After removal of fungus.			
	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch present as reducing sugars.	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch present as reducing sugars.	Hydrolysis of starch by enzym powder.
a.....	43.62	0	43.62	29.47	10.69	18.78	9.10
b.....	No starch in solution.	112.19	0	.....	36.56	0	.78
c.....	153.60	112.64	40.96	75.15	33.97	41.18	.39
d.....	449.55	272.56	176.99	182.25	144.00	38.25	11.54

These results accord in general with those of the previous experiments, series a and d having the greatest hydrolyzing power and b and c the least.

EXPERIMENT 4.—The foregoing experiment was repeated, the solutions being made to contain roughly the same amount of glucose and starch. The hydrolysis of starch by the enzym powder was determined by using 0.25 gm. enzym powder in 100 cc. of a 0.5 per cent starch paste solution and hydrolyzing 18 hours at 40° C. (Table XVI.)

TABLE XVI.—*Amount of reducing sugars and starch in the solutions before and after the growth of the fungus; also the products of hydrolysis of starch by enzym powder*

[Expressed in milligrams per 10 cc. of solution]

Series.	Before inoculation.			After removal of fungus.			
	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch as reducing sugars.	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch as reducing sugars.	Hydrolysis of starch by enzym powder.
a.....	31.275	No sugar used.	31.275	14.30	2.86	11.44	7.15
b.....	No starch.	105.41	0	.....	45.35	0	1.27
c.....	144.680	108.68	36.000	86.32	67.60	18.72	1.24
d.....	524.000	300.37	223.630	107.90	107.90	0	22.94

EXPERIMENT 5.—This experiment was conducted in the same way as experiments 3 and 4. The amyloclastic power of the enzym was determined as in experiment 4. (Table XVII.)

TABLE XVII.—*Amount of reducing sugars and starch in the solution before and after the growth of the fungus; also the hydrolysis of starch by enzym powder*

[Expressed in milligrams per 10 cc. of solution]

Series.	Before inoculation.			After removal of fungus.			
	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch as reducing sugars.	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch as reducing sugars.	Hydrolysis of starch by enzym powder.
a.....	47.97	0	47.97	No starch left.	9.23	0	26.19
b.....	No starch used.	122.20	0	No starch used.	33.93	0	5.78
c.....	213.98	120.90	93.08	124.80	44.72	80.08	4.42
d.....	508.56	298.48	210.08	217.62	144.30	73.32	41.08

An examination of the foregoing results shows a clear case of the regulatory influence of the culture medium on the quantitative secretion of amylase. In every case where starch (series a) alone was used as the source of carbon the enzym powder hydrolyzed several times as much starch in a corresponding length of time as when glucose alone (series b) or in combination with starch (series c) was used. On the other hand, the enzym powder from sweet potato bouillon (series d), which always contained reducing sugars and starch and probably other carbohydrates, hydrolyzed considerably more starch than the powder from the a series. This exception is hard to explain, since it was obviously impossible to determine the exact composition of sweet potato bouillon. That it was a better medium for the growth of the fungus was quite evident. The quantity of felt was always greater than in any of the other series. The growth in the a series was likewise better than in either the b or c series, starch alone appearing to be a better source of carbon than glucose alone or in combination with starch.

These results seem to indicate that within the limits of these experiments the solution which is best for the growth of the fungus is likewise best for the secretion of amylase, regardless of the source of carbohydrates. It is probable that it is not so much the source of the carbohydrate which influences directly the quantitative production of the enzym as the influence it has upon the growth of the fungus on which the secretion of the enzym depends.

INFLUENCE OF THE AGE OF THE MYCELIUM WHEN REMOVED FROM THE CULTURE ON THE PRODUCTION OF AMYLASE

It was shown by Dox, Young, and others that the greatest amount of enzym is contained in the mycelium at about the beginning of the fruit-

ing period. So far as the writer is aware, this fact has not been determined for *Rhizopus tritici*, and it was with the view of verifying it for this fungus alone that comparative tests were made. In the experiments carried out by the writer two different culture media were used—namely, sweet potato bouillon and a modification of Czapek's nutrient solution with a 0.5 per cent starch paste as a source of carbon. In the former case the mycelium was removed from one set of flasks 3 days after inoculation, when fruiting was just beginning. The mycelium was removed from the other set of flasks 10 days after inoculation. The difference in reducing power in this case was not large.

On the other hand, when the Czapek's modified solution was employed, the mycelium removed 5 days after inoculation (when just beginning to fruit) hydrolyzed considerably more starch in a given length of time than the mycelium removed 10 days later.

SUMMARY

(1) A vigorous starch-splitting enzym is secreted by *Rhizopus tritici*. While some of the enzym is retained in the mycelium of the fungus, a portion of it diffuses out into the substratum. The diffusion into the culture medium begins soon after the substratum is inoculated, as was shown by some of the experiments in which reducing sugars appeared after 2 days in a nutrient solution with starch as the only source of carbon. The reducing sugars in such a medium accumulate in excess of the needs of the fungus.

(2) The enzym is able to act on raw sweet potato and Irish potato starch but much less energetically than on starch paste.

(3) The dried mycelium may be stored for several months at a temperature of from 9° to 35° C. without much deterioration, but at 60° it gradually becomes weaker.

(4) The optimum temperature for the digestion of starch is about 45° C. Above and below this temperature the amount of hydrolysis becomes less, and at 60° it is completely destroyed in 100 hours.

(5) If glucose is added to a system the hydrolysis of starch paste is retarded. The quantity of glucose added does not seem to influence the results. With a constant amount of enzym powder the total reducing sugars formed in a solution of starch paste increases with the increase in the volume of the solution up to a certain point and then decreases.

(6) An end point in the hydrolysis of the starch is not reached without altering the equilibrium of the system. This was done by changing the temperature and diluting the solution. If judged by the iodin test an end point was obtained, but a quantitative determination of the reducing sugars did not account for all the starch. It is probable that in this case some of the products of the hydrolysis were dextrins which were not accounted for as either starch or reducing sugars.

(7) When the enzym is in suspension some of it is removed by filtering through Whatman chemically prepared filter paper.

(8) The temperature at which the fungus is grown has a marked influence on the production of intercellular amylase. With an equal weight of enzym powder it was found that mycelium grown at 9° C. hydrolyzed about four times as much starch in the same length of time as mycelium grown at 40°. The enzym powder of mycelium grown at 29° was intermediate between the other two. At these three temperatures the best growth of the fungus was made at 29° and the poorest at 9°.

(9) The results of these investigations show that there is a "quantitative regulation" of the enzym. The hydrolyzing power of the mycelium grown on Czapek's modified nutrient solution was much greater when starch alone was used as a source of carbon than when glucose alone or in combination with starch was employed. On the other hand, if grown on sweet potato bouillon, which contains both starch and sugars, a unit weight of the mycelium will hydrolyze more starch than when grown on any of the other combinations. The vigor of growth of the fungus was correlated with the hydrolytic power of the enzym powder. The results seem to indicate that it is not so much the source of the carbohydrate which influences the quantitative production of the enzym as it is the influence which it has on the growth of the fungus on which the secretion of the enzym depends.

(10) The enzym powder of young mycelium just beginning to fruit was more active than the enzym from old mycelium.

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A COMPARATIVE STUDY OF THE COMPOSITION OF THE SUNFLOWER AND CORN PLANTS AT DIFFERENT STAGES OF GROWTH

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INTRODUCTION

The sunflower plant is gaining recognition as a silage crop in certain of the northwestern States where climatic or soil conditions are not always favorable for the maturing of corn for silage purposes. In some sections also there is a growing sentiment that sunflower silage offers a more profitable feed than corn silage, because of the greater yield that may be obtained per acre.

The Dairy Division is making an investigation of sunflower silage. This paper, which is the first of a series, presents the results of a study of the chemical composition of the sunflower plant at several different and distinct stages of its growth as compared with that of corn grown under similar conditions. The purpose of the study is to assist in selecting the proper stage of maturity for ensiling.

The investigation of the corn plant was made partly as a basis on which to study the sunflower plant and partly in connection with another investigation, the results of which will be published in a paper having to do with the fermentation of corn in the silo.

HISTORICAL REVIEW

Numerous analyses of the sunflower plant have been published from time to time. In some cases these have represented the whole plant, but more often only the head or the seed. No record of any study of the composition of the plant at different stages of growth has been found. On the other hand, there have been several such studies, more or less complete, made of the corn plant. Some of these will be briefly reviewed.

Roberts (*5*)[1] selected periods of growth (1) when the plants were coming into bloom, (2) when approaching roasting-ear condition, and (3) when most of the ears were out of the milk. Basing his figures on the dry matter, he found that the percentage of protein decreased from the first period to the last, while the percentage of carbohydrates increased.

Ladd (*3*) concludes that the nitrogen steadily diminishes throughout the period of growth, while the sugars rise and fall. The starch falls slightly during the earlier stages and then rises rapidly until the plant reaches maturity.

[1] Reference is made by number (italic) to "Literature cited," p. 792–793.

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Morse (*4*) analyzed samples representing four stages of growth and reached the same conclusions, with respect to the protein and carbohydrates, as the other investigators.

Perhaps the most elaborate study of the subject was made by Jones and Huston (*2*). Their study included the whole plant as well as the stalks, leaves, and ears taken separately. Unfortunately their figures for the whole plant are based upon yield per acre and so can not be compared with those of the other investigators or with ours.

EXPERIMENTAL WORK

The crops for the experimental work were grown in a section of the field at the Dairy Division Experiment Farm at Beltsville, Md., usually devoted to silage corn. The preparation of the soil, the planting, and cultivating were done under the supervision of T. E. Woodward, farm superintendent.

The sunflower plants were of the variety known as Giant Russian, and the corn was Boone County White. The sunflower plants thrived well in this soil (Bibb silt loam), reaching a height in many cases of 10 and 12 feet.

In dividing the growing period of the corn plant into stages, more or less arbitrary points must be taken. It is quite useless for the purpose to select plants by their age or height, for it is easily possible to find at any one time within a comparatively small area plants of the same height and age at entirely different stages of maturity. Up to the time of tasseling, however, there are no easily recognized guides except height. From that time until the plant is fully mature there are certain and fairly distinct points that can be selected, based on the condition of the silk and ears.

The task of selecting stages of growth of the sunflower plant offers more difficulty, and it is quite impossible to divide it into anything like as sharply defined stages as in the case of the corn plant. We endeavored to differentiate the stages first by the height and later by the condition of the flower and seed, but at best these points are very arbitrary.

The difficulties in selecting representative samples of whole plants for chemical analysis are obvious. The plan we followed was to go through a small area of the field and select from 6 to 20 plants of the proper stage of growth and as nearly the same size and conformity as possible. These were carefully wrapped in a specially prepared waterproof cloth and taken immediately to the laboratory, where they were cut into 1-inch lengths with a hand-power feed cutter.

A 1-kilogram subsample was weighed out and dried in the steam closet for the determination of starch. The remainder was ground to a pulp in a power meat grinder, and a subsample was taken for moisture, albuminoids, and total-protein determinations. A further subsample

was weighed out, from which the alcoholic extract of the pulp was prepared according to the method described by Swanson and Tague (*6*). Aliquot portions of the alcoholic extract were used to determine total and reducing sugars according to the gravimetric cuprous-oxid method of Walker and Munsen (*7, p. 241*).

Moisture was determined on a 5-gm. sample of the pulp by drying to constant weight in a reduced pressure water-jacketed oven. The subsample dried in the steam closet was ground to pass a 40-mesh sieve, and starch was determined on the air-dry sample by the diastase method with subsequent acid hydrolysis (*1, p. 110*).

Tables I and II give the results of the chemical work on the whole plants. The figures for total protein, albuminoid protein, reducing sugars, nonreducing sugars, and starch are based on the dry matter.

TABLE I.—*Composition of sunflower plant at different stages of growth*

Stage of maturity.	Moisture in fresh material.	Dry matter.	Moisture-free basis.				
			Total protein.	Albuminoid protein.	Reducing sugars.	Nonreducing sugars.	Starch.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
3 feet high	84.87	15.13	8.59	8.00	12.36	19.08	0.63
6 feet high	86.02	13.98	8.01	7.37	18.95	15.63	4.61
First flower	84.09	15.91	7.04	6.35	15.96	8.43	4.34
Rays ready to fall	83.90	16.10	9.44	7.89	13.23	3.01	.20
Rays dry and partly fallen	75.58	24.42	6.80	6.22	8.96	1.40	.84
Rays all fallen	74.37	25.63	7.03	6.09	6.99	.89	1.66
Seeds hard and mature	69.68	30.32	5.90	5.04	4.15	1.47	1.90

TABLE II.—*Composition of corn plant at different stages of growth*

Stage of maturity.	Moisture in fresh material.	Dry matter.	Moisture-free basis.				
			Total protein.	Albuminoid protein.	Reducing sugars.	Nonreducing sugars.	Starch.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
3 feet high	84.21	15.79	11.14	10.26	14.69	2.73	1.52
4½ to 5 feet high	85.14	14.86	9.42	8.14	16.69	3.23	1.66
Just tasseling	81.65	18.35	9.90	6.59	13.13	1.85	1.29
Just silking	81.56	18.44	8.95	6.73	18.23	1.30	.86
Kernels forming	81.20	18.80	8.99	6.38	20.37	5.44	3.45
Milk stage	77.60	22.40	8.97	6.30	17.59	4.51	2.87
Silage stage (one-half milk, one-half glazed)	68.69	31.31	7.31	6.23	10.03	2.81	24.00
All glazed	64.22	35.78	6.32	5.62	8.50	5.39	24.78
Ready to shock	59.79	40.21	7.09	6.14	7.71	2.73	21.66

DISCUSSION OF RESULTS

In studying the tables it should be borne in mind that the figures represent percentages based on the plants themselves and have no bearing on the yield of the various constituents per unit of area. For example, the proteids decline in percentage as the plant grows. This does not mean, of course, that the amount of the proteids per given area decreases, but rather that as the plant grows and increases in weight the proteids do not increase in the same ratio.

Too much importance must not be placed on slight differences in composition from stage to stage of growth. Because of the difficulties in sampling whole plants, small differences due to unavoidable errors are to be expected, and conclusions are safest when drawn from the general trend of the results rather than from particular figures.

Considering the sunflower plant first, it will be noted that the dry matter steadily increases as the plant grows older. This, of course, is what would be expected, but the fact is rather surprising that, even after the rays had all fallen and the seeds had become dry and mature, the plant still contained more moisture than the corn contained at the time it was ready for the silo.

The proteids, both total and albuminoid, show a tendency to decline as growth proceeds. This is somewhat contrary to what might be expected from the highly nitrogenous character of the seed.

The reducing sugars rise and then gradually decline. The nonreducing sugars steadily and rapidly decline throughout the whole period of growth. In the first stage there is one and one-half times as great a quantity of nonreducing sugars present as reducing sugars. This relation, however, is quickly changed, and in the last stage there is nearly three times as much of reducing sugars present as nonreducing. The percentage of starch is small, rising and falling with no apparent relation to the change in percentage of the sugars.

Turning now to the corn plant, it will be noted, as would be expected, that the dry matter steadily increases as the plant grows older. The proteids, both total and albuminoid, decline slowly but quite regularly. The sugars, both reducing and nonreducing, rise and fall but have an upward trend until the kernels begin to mature, when there is a sharp drop, accompanied by a sudden increase in the starch. This is at the stage when the plant is storing starch in the kernels and is the stage usually selected for ensiling. The ratio of reducing and nonreducing sugars changes, but within a somewhat narrow range. The reducing sugars always greatly exceed the nonreducing. The starch rises and falls up to the stage when the kernels begin to mature. Between the milk stage and what may be called the silage stage the starch increased from 2.87 per cent to 24 per cent.

Comparing the sunflower and the corn plants, it will be noted that the chief difference in the constituents studied lies in the amount and char-

acter of the carbohydrates. Although no part of the present experiment, silage was made of the sunflower plant at different stages of maturity, and it was found that silage made from plants at the stage when the rays were dry and partly fallen was excellent in quality. Comparing the plant at this stage with the corn plant at the silage stage, it will be seen that the starch and sugars combined constitute 11.2 per cent of the dry matter in the former, of which only about one-fifteenth is starch, while the combined starch and sugars in the dry matter of the latter constitute nearly 37 per cent, two-thirds of which is starch.

There is no great difference in the percentage of proteids in the dry matter of the two plants, but it is slightly in favor of the corn plant.

In selecting the best stage of maturity of a plant for ensiling, several things must be taken into consideration. In general the stage must be selected that promises the largest yield of food constituents in the silage. This stage is not necessarily the one when the plant itself has the maximum amount of food constituents. The moisture content of a plant, judging by the behavior of the corn plant when ensiled, plays an exceedingly important rôle. When silage is made from the corn plant having a high moisture content there is a downward seepage of the juice, carrying with it valuable food material. If the silo is tight this juice waterlogs the bottom layer, rendering it unfit for feeding. If the silo is not tight the juice leaks out and is lost altogether. Moreover, high moisture in the plant is usually associated with high-acid silage. On the other hand, a plant that has too low a moisture content is difficult to pack closely enough to eliminate the air spaces that cause spoilage. Silage produced from such plants is dry and lacks palatability.

Another point that should not be lost sight of is, of course, the yield per acre. This point, aside from the high moisture content, would bar out the three earlier stages of the sunflower plant. The fourth stage is still too high in moisture. The last stage contains nearly 70 per cent of moisture.

From the moisture content alone the sunflower plant at this stage should make good silage, but here another factor must be taken into consideration. The sunflower plant at this stage has lost some of its leaves. The outer part of the stalk has become so hard and woody that it would be difficult, if not impossible, to pack it closely enough to prevent spoilage. This eliminates all but two stages, the one when the rays are dry and partly fallen and the other when all the rays have fallen. These stages are close together, and judging from the chemical composition there is but little choice between the two.

There is but little difference in percentage between the total proteids and albuminoid proteids in the sunflower plant at these stages and the corn plant at the silage stage. The chief differences, as discussed in another paragraph, lie in the sugars and starch.

SUMMARY AND CONCLUSIONS

A study was made of the chemical composition of the sunflower and corn plants at different stages of growth.

The dry matter in each increased gradually and consistently throughout the entire period of growth.

There is no great difference in the percentage of proteids in the two plants, but it is slightly in favor of the corn plant.

The reducing and nonreducing sugars in the sunflower declined somewhat irregularly but persistently during the growth of the plant. In the first stage there was about one and one-half times as much nonreducing sugars present as reducing sugars. This relation was quickly changed, and in the latter stages the reducing sugars greatly exceeded the nonreducing.

The percentage of starch in the sunflower is small and rises and falls irregularly throughout the growth of the plant.

The reducing and nonreducing sugars in the corn plant rise and fall but with a marked upward trend during the growth of the plant until the stage is reached where the kernels are maturing, when a sudden drop occurs. The percentage of reducing sugars is always far in excess of the nonreducing sugars.

The starch rises and falls until the kernels are maturing, when a sudden rise occurs.

The chief difference between the two plants at the silage stage lies in the amount and character of the carbohydrates.

From the results obtained in this study it would seem that the best stage of maturity for ensiling the sunflower plant is when the rays of the flower have become dry and are falling.

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EVALUATION OF CLIMATIC TEMPERATURE EFFICIENCY FOR THE RIPENING PROCESSES IN SWEET-CORN

By CHARLES O. APPLEMAN, *Plant Physiologist*, and S. V. EATON, *former Assistant Plant Physiologist, Laboratory of Plant Physiology, Maryland Agricultural Experiment Station*

INTRODUCTION

Green sweetcorn for table use or packing into cans is picked while the ripening processes are actively in progress. Since these processes greatly change the chemical composition of the corn, it is obvious that the ears must be picked as nearly as possible at the stage of ripening that will furnish the most desirable quality. There is some difference of opinion in regard to the chemical composition that gives the most desirable quality to sweetcorn, especially for packing into cans. Attention is usually focused upon sugar content, as sweetness is a desirable quality of sweetcorn and, morever, the flavor appears to be associated with the sugar content. This relationship may be merely a parallelism, but it is certainly true that corn acquires a decided flat taste after the sugar is reduced to low content either on the stalk or in storage. The foregoing statement does not necessarily apply to naturally low sugar content in certain varieties or to the same variety grown under different conditions.

The percentages of starch and crude fiber are claimed by some to be of equal if not of even greater importance than the sugar content. The percentage of starch must be sufficiently high to give body to the corn, while the amount of crude fiber must be kept as low as possible. Since the starch and crude fiber increase at the expense of the sugar, the most desirable stage for picking corn would seem to be a wise compromise between sugar content and other constituents.

The present paper deals with the chemical changes in sweetcorn during ripening and the effect of climatic temperature on rate of these changes. An attempt has also been made to evaluate the climatic temperature efficiency for these processes and to make the results of some practical value as a guide for picking corn in different localities and in different seasons in the same locality.

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In this study a distinction has been made between the ripening and the maturing processes. The corn is considered ripe when the growth of the kernels ceases and the chemical changes in the corn have nearly attained equilibrium positions—that is, it is ripe at the time after which the ratios of the various constituents change very slowly and very little. The maturing of corn consists essentially in the loss of water; therefore, the rate at which corn matures depends largely upon the climatic conditions which control evaporation.

CHANGES IN CHEMICAL COMPOSITION OF SWEETCORN DURING RIPENING

Stowell's evergreen corn grown from home-selected seed furnished the material for this study. For each experiment 50 ears representing as nearly as possible the same stage of ripening were carefully selected in the center of the field. These ears were numbered consecutively and designated as being in the premilk stage. The husks were not yet firm, and the silk was still green or red for about ½ inch beyond the tip of the husks. The remainder of the silk was, as a rule, brown but not dry. The kernels were inspected through a small longitudinal slit in the husks which was afterwards carefully closed and tightly held with a rubber band. The spikelets were still evident, the kernels small and spherical, and the exudate was opalescent or cloudy but not milky. This is about the earliest stage of ripening that will furnish sufficient kernel material from a single ear for sampling.

Samples for analyses were taken at 10 o'clock a. m. every other day during the ripening period. In order that the rate of change in chemical composition during each succeeding 48-hour period might be determined by comparing analyses from the same ear, as well as analyses from different ears, the following procedure was adopted: Samples of three rows of kernels each were removed from ears 1 and 2. The husks were then carefully brought back to place and held with rubber bands. After 48 hours a second pair of like samples was taken from the opposite sides of ears 1 and 2. At the same time the first pair of samples was removed from ears 3 and 4. At the end of the second 48-hour period the second samples were removed from ears 3 and 4 and the first samples from ears 5 and 6. This overlapping method of sampling was continued throughout the ripening period.

The treatment of the samples and the methods for the carbohydrate determinations have been described in a previous paper.[1] The methods for fat, crude fiber, and total nitrogen were essentially those of the Official Agricultural Chemists.[2]

[1] APPLEMAN, Charles O., and ARTHUR, John M. CARBOHYDRATE METABOLISM IN GREEN SWEETCORN DURING STORAGE AT DIFFERENT TEMPERATURES. *In* Jour. Agr. Research, v, 17, no. 4, p. 137–152. 1919.

[2] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at ends of chapters.

Table I shows the changes in chemical composition of the solids in the corn during a typical ripening period. It was found that the rate of ripening was fairly uniform in all the ears selected for the experiment. Therefore the determinations from the four samples taken at each sampling period were averaged instead of the first and second samples from the same ears being compared, as was originally intended. Each percentage in the table, except the first set, represents an average of four determinations. The averages for the first date include the determinations from the first samples of ears 1 and 2. The averages for the succeeding dates include the determinations from the first samples of two ears and the second samples of the two ears that furnished the first pair of samples on the previous date. The removal of the first sample from an ear does not affect the rate of ripening in the kernels on the remaining half of the ear if the husks are closed tightly and held in place.

TABLE I.—*Changes in composition of sweetcorn during ripening*

[Calculated as percentages of dry weight]

Date.	Starch.	Cane sugar.	Reducing sugars.	Fat.	Crude fibre.	Total nitrogen.	Protein (total N×6.25).
Aug. 3.....	18.36	19.55	20.07	2.97	7.92	3.33	20.81
5.....	25.20	21.85	13.93	4.04	6.37	3.08	19.25
7.....	35.73	24.57	9.45	3.99	4.63	2.45	15.31
9.....	45.42	18.75	5.43	4.44	2.58	2.09	13.06
11.....	56.89	11.59	3.01	4.81	2.62	2.14	13.37
13.....	57.23	9.55	2.64	5.25	2.81	2.01	12.56
15.....	58.91	8.32	2.24	5.05	2.35	2.03	12.70
17.....	59.15	7.86	1.97	5.01	2.59	2.10	13.12
19.....	60.41	5.85	1.77	6.01	2.30	2.20	13.75

The chief changes in the percentage composition of the solids in the corn during ripening consist in the depletion of sugars and the increase in starch. In the very early stages the reducing sugars predominate but very rapidly decrease as ripening proceeds. The percentage of cane sugar increases until a maximum is reached and then decreases as the starch increases. The reducing sugars predominate at the stage of highest total sugar content; therefore this stage does not necessarily coincide with the stage of greatest sweetness, as the reducing sugars are not nearly as sweet as cane sugar. The highest content of the latter sugar is the stage of greatest sweetness. The changes in the percentage of fat, crude fiber, and total nitrogen occur during the very early stages of ripening. For the remainder of the ripening period these percentages remain fairly constant.

The formation and storage of starch is the chief process occurring in the kernels during ripening. This is the resultant of a number of complex processes in the plant, but it seems safe to conclude that the rate of starch synthesis in the kernels is the controlling factor for several supplementary processes in the ripening of the corn. For example, the rate

of movement of soluble carbohydrates from the stems and cob to the kernels and the rate of hydrolysis of cane sugar in the kernels are both controlled by the rate of starch formation. Most of the starch that is stored in the kernels during ripening is formed from carbohydrates already stored in the stem and cob when kernel formation begins. The intensity of respiration does not change the ratios of the different carbohydrate constituents in the ripe corn. The carbohydrate transformations being reversible, their final equilibrium positions are maintained.

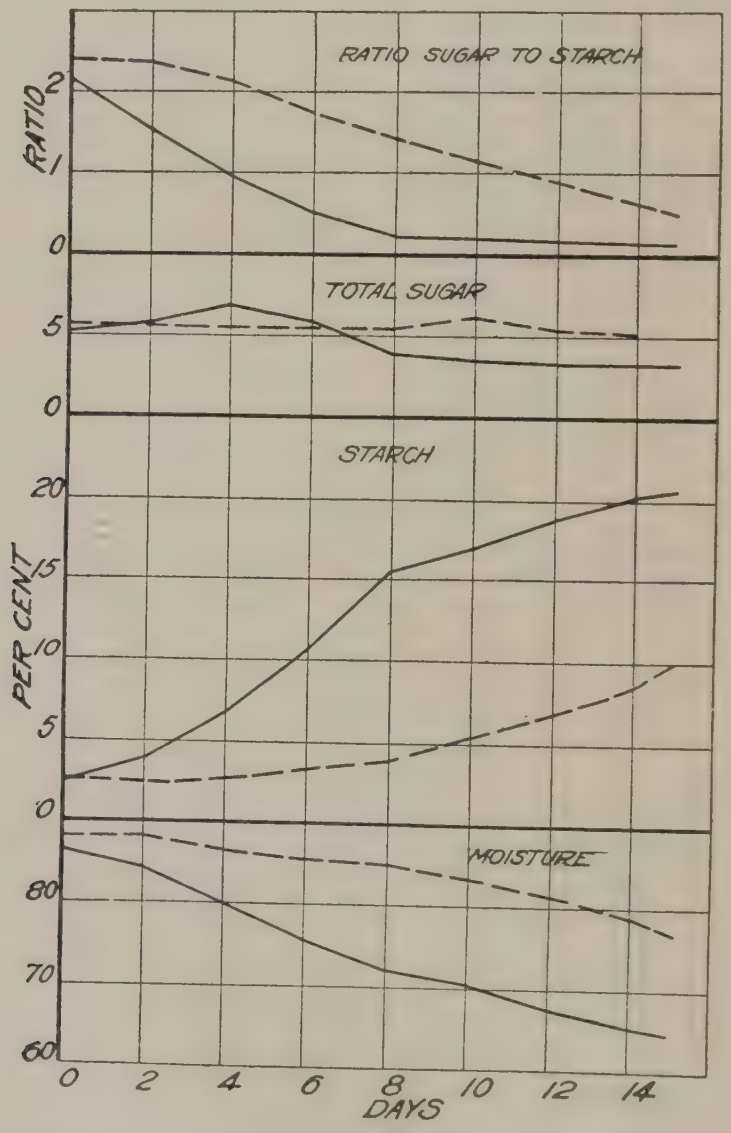


FIG. 1.—Comparison of early and late crops of sweetcorn in respect to changes in percentage composition in equal lengths of time. Early crop (Aug. 3 to 18) indicated by solid lines. Late crop (Sept. 20 to Oct. 5) indicated by broken lines.

EFFECT OF SEASON ON THE RATE OF RIPENING

Two crops of corn from the same source of seed were planted so that the first crop would ripen in August and the second in the cool autumn. In order to compare the ripening rates of the early and late crops, it was necessary to find a measure of the rate of ripening. The decrease in the

ratio of total sugar to starch was adopted for this purpose. Table II and figure 1 show the changes in percentage of moisture, total sugar and starch, and also the changing ratio of sugar to starch in equal times for the two seasons, starting with the same stage of ripening in both cases. By comparing these ratios it will be noted that the late crop required 15 days to reach the same stage of ripening as the early crop reached in 6 days. In other words, the rate of ripening was two and one-half times faster in the early crop than in the late crop. During this period of ripening the starch content in the early crop increased from about 2.5 per cent to 10.5 per cent, and in the late crop from about 2.7 per cent to 10 per cent. At the end of this ripening period the sugar to starch ratios were 0.556 and 0.500, respectively, and the chemical composition was such that it probably represented the best edible stage. By the nail test the corn was in the typical milk stage, but a subsequent paper will show that the chemical composition of the corn changes considerably during the so-called milk stage.

TABLE II.—*Comparison of early and late crops of sweetcorn in respect to changes in percentage composition in equal lengths of time*

Time from first examination.	Early crop.				Late crop.			
	Moisture.	Total sugars.	Starch.	Ratio of sugar to starch.	Moisture.	Total sugars.	Starch.	Ratio of sugar to starch.
Days.								
0	86.55	5.39	2.47	2.187	88.27	6.13	2.72	2.300
2	84.21	5.90	3.98	1.544	88.83	5.69	2.32	2.459
4	80.63	6.89	6.92	.868	86.97	5.78	2.86	2.168
6	75.89	6.09	10.95	[a].556	85.56	5.53	3.39	1.747
8	72.05	4.21	15.90	.264	85.21	5.56	3.85	1.448
10	70.47	3.75	16.93	.219	83.80	6.30	5.48	1.164
12	67.78	3.50	18.98	.183	81.56	5.62	6.90	.879
14	65.51	3.55	20.42	.170	79.26	5.26	8.71	.673
15	64.98	3.02	20.94	.149	77.69	5.08	10.09	[b].500

[a] Same stage of ripening as late crop on fifteenth day.
[b] Same stage of ripening as early crop on sixth day.

EVALUATION OF CLIMATE TEMPERATURE EFFICIENCY FOR THE RIPENING PROCESSES IN SWEETCORN

Since both the early and late crops of corn were grown from the same source of seed and on the same type of soil, the great difference in the rate of ripening must have been due to the different climatic conditions which prevailed during the ripening periods. Of the climatic conditions, temperature was the most important variable. The averages of the hourly mean temperatures for the ripening periods of the early and late crops were 83° and 65° F., respectively. The ripening processes being either chemical or dependent upon chemical processes, the prevailing temperatures for the two periods would be expected to have a very different

influence on the rate of ripening. But these ordinary temperature readings do not furnish a basis for a quantitative comparison of the temperature efficiency in reference to these processes.

Various methods have been proposed for interpreting the observed climatic temperatures in different localities and for different seasons in the same locality, with reference to plant growth. Three of these methods were applied to the fairly definite set of physico-chemical processes involved in the ripening of sweetcorn. The first method employed was one of direct temperature summation, similar to that described by MacDougal.[1]

The integration was performed, with a planimeter, upon thermograph records. The area between the 40° F. line and the pen tracing for each day of the two ripening periods was first measured. Then the mean temperature for each hour of a chosen day was computed from a thermograph record, and 40 was subtracted from each hourly temperature.[2] The sum of these results divided by the planimeter reading for the same day gave a factor by which the planimeter reading for any 24-hour period could be converted into hour-degree units of effective temperature. The total number of hour-degree units was computed for the 6- and 15-day ripening periods of the early and late crops, respectively. These units express both the intensity and duration aspects of the temperature factor. The adoption of the 40° as the starting point for the temperature summations was based upon the facts that carbohydrate changes are chiefly involved in ripening and that carbohydrate transformations in green corn during storage are extremely slow below this temperature.

The results of the direct temperature summations given in Table III show a slightly greater total number of hour-degree units of effective temperature in favor of the late crop. Stevens and Higgins[3] have shown that the temperature of green corn on the stalk in the shade is nearly that of the air, while in the sun it is often above that of the air. The period of ripening for the early crop here considered was characterized by high temperature and clear days, while the ripening period of the late crop contained 2.5 times as many days, many of which were cloudy. Since the temperature records from which the units of effective temperature were computed were taken in an instrument shelter, the sum of the hour-degree units for the early crop is probably a little less than actually required.

Livingston and Livingston,[4] realizing the need of some fundamental principle of physiology upon which to base the value of temperature

[1] MacDougal, D. T. THE TEMPERATURE OF THE SOIL. *In* Jour. N. Y. Bot. Garden. v. 3, no. 31, p. 125–131, fig. 19–21. 1902.

[2] The thermograph records were furnished by Dr. Earl S. Johnston of the Laboratory of Plant Pathology, Maryland Agricultural Experiment Station.

[3] Stevens, Neil E., and Higgins, C. H. TEMPERATURE IN RELATION TO QUALITY OF SWEETCORN. *In* Jour. Agr. Research, v. 17, no. 6, p. 275–284, 1 fig. 1919. Literature cited, p. 283–284.

[4] Livingston, Burton Edward, and Livingston, Grace Johnson. TEMPERATURE COEFFICIENTS IN PLANT GEOGRAPHY AND CLIMATOLOGY. *In* Bot. Gaz., v. 56, no. 5, p. 349–375, 3 fig. 1913.

summations, were the first to apply velocity coefficients to the study of effective climatic temperature conditions for plant growth. Upon the basic assumption that the growth rate is unity at 40° F. and that it doubles for each rise of 10° C. (18° F.), they deduced temperature efficiency values corresponding to temperatures, in whole numbers, from 40° to 99° F. These efficiency values are spoken of as exponential indices. Since the rate of the carbohydrate changes in corn after it is pulled has a temperature coefficient of about 2 for a range of temperature beyond the limits of the climatic temperature for either ripening period, and since the chief process during ripening is the conversion of sugar into starch, the exponential indices would be expected to furnish the best criteria of the temperature efficiency for the ripening processes in sweetcorn. In Table III are given the sums of the exponential indices corresponding to the daily mean temperatures of each ripening period under consideration, as well as the average daily index for each period. The average daily index for the early season is 2.5 times greater than that of the late season. If these indices furnish an approximate criterion of the temperature efficiency for ripening of sweetcorn, the ripening should have proceeded 2.5 times faster during the early ripening period than during the late ripening period. The experimental data show that this was actually the case; the late season required 15 days to carry the corn to the same stage of ripening that required only 6 days in the early season, a time ratio of 2.5.

More recently Livingston [1] has derived a new set of temperature indices which he terms physiological indices, since they are based upon Lehenbauer's actual measurements of the hourly rate of elongation of the shoots of seedling maize plants. For the sake of comparison these indices for the two ripening periods are also given in Table III, but it will be seen at once that they do not furnish even an approximate criterion of the temperature efficiency for the ripening processes in sweetcorn. This may be at least partially explained by the fact that, for the processes under consideration, the principle of Van't Hoff and Arrhenius seems to hold for rather a wide range of temperature, while in the elongation of maize shoots it holds only for a range of temperature from about 20° to 30° C.

[1] LIVINGSTON, Burton Edward. PHYSIOLOGICAL TEMPERATURE INDICES FOR THE STUDY OF PLANT GROWTH IN RELATION TO CLIMATE CONDITIONS. *In* Physiol. Researches, v. 1, no. 8, p. 399-420, 4 fig. 1916. Literature cited, p. 420.

TABLE III.—*Temperature indices in relation to ripening of sweetcorn*

Crop.	Time between premilk and best edible milk stages.	Hour-degree units.	Exponential indices.		Physiological indices.	
			Sum.	Average.	Sum.	Average.
	Days.					
Early	6	6,425	31.81	5.3020	640	107.0
Late	15	7,393	32.22	2.1458	319	21.3

EXPONENTIAL INDICES AS A BASIS FOR AN APPROXIMATE PREDICTION OF THE RATE OF RIPENING IN SWEETCORN

Since the rate of ripening appears to be inversely proportional to the exponential indices, the proportions

$$6 : x :: y : 5.3020$$
$$2 : x :: y : 5.3020$$

furnish a basis for an approximate prediction of the number of days in different localities and for different seasons in the same locality required for corn to pass from the premilk stage to the best edible milk stage, and also the maximum number of days that the corn may be expected to remain in this condition. The first term of the first proportion is the number of days actually required for an early crop to pass from the premilk to the best edible stage, or from a starch content of about 2.4 per cent to one of 11 per cent. The first term of the second proportion is the maximum number of days that the corn of the early crop here considered remained in the best edible condition. The last term of the proportions is the average of the exponential indices corresponding to the daily mean temperatures for the 6-day period. By substituting for y in these proportions the average of the exponential indices derived from the normal daily mean temperatures for any season of any locality, the value of x in the first proportion gives the approximate number of days on the average that will be required for the corn to pass from the premilk to the best edible condition. The value of x in the second proportion gives the number of days that the corn may be expected to remain in this condition.

Table IV gives the values of x for the usual ripening seasons of four sweetcorn localities which show considerable variation in the normal mean temperature for the ripening periods. In this calculation, the normal mean temperatures calculated by Bigelow[1] were employed.

[1] BIGELOW, F. H. THE DAILY NORMAL TEMPERATURE AND DAILY NORMAL PRECIPITATION OF THE UNITED STATES. U. S. Dept. Agr. Weather Bur. Bul. R, 186 p. 1908.

TABLE IV.—*Comparison of the rates of sweetcorn ripening in different localities, based upon the exponential indices corresponding to the normal mean temperatures of the ripening seasons*

Locality.	Ripening season.	Time between pre-milk and best edible milk stage.	Length of time in best edible stage.
		Days.	*Days.*
Charleston, S. C.	June 17 to 31	7.0	2.5
	July 1 to 15	6.5	2.0
Baltimore, Md.	Aug. 1 to 15	8.0	2.5
	Aug. 16 to 31	8.5	3.0
	Sept. 1 to 15	9.5	3.0
	Sept. 16 to 30	11.5	4.0
	Oct. 1 to 15	14.0	5.0
New Haven, Conn.	Aug. 1 to 15	9.5	3.0
	Aug. 16 to 31	10.5	3.5
Portland, Me.	Sept. 1 to 15	14.0	4.5
	Sept. 16 to 30	16.0	5.5

The results given in Table IV are simply the average expectations, calculated for a 20-year period. If the mean temperature for a particular season deviates to any considerable extent from the normal mean, the rate of ripening for this season will be greater or less, depending upon the direction of the deviation, than that calculated from the normal mean temperature. In order to test the possible magnitude of deviation from the average expectation, the ripening rates were calculated for the highest and lowest mean August temperature at Baltimore from 1871–1918. These results together with those calculated from the normal mean August temperature for the same period are given in Table V. Data were not available from which to derive the exponential indices corresponding to the daily mean temperatures for the month as was done in calculating the data from normal mean temperatures given in Table III. However, the results suffice to indicate that for the most extreme seasons the number of days required for the two periods of ripening under consideration would not vary more than a day or two in either direction from the calculated average. If the particular season in question is unusually hot, one day would have to be subtracted from the average prediction. If, on the other hand, the season is unusually cool, one day would have to be added to the average expectation. This applies particularly to Maryland conditions.

In making the foregoing predictions it was assumed that most of the ears of a given crop will ripen at practically the same rate. This was found to be true in the experimental crops grown from home-selected seed. For canning purposes it is essential to use seed that will insure the maximum uniformity in ripening.

TABLE V.—*Rate of sweetcorn ripening during the month of August, calculated from Baltimore temperatures*

Temperature.	Exponential index.	Time between pre-milk and best edible stage.	Length of time in best edible stage.
		Days.	*Days.*
Normal mean, 75.3° F.	3.8480	8.3	2.7
Highest monthly mean, 1871–1918, 80.0° F.	4.6662	6.8	2.3
Lowest monthly mean, 1871–1918, 72.0° F.	3.4283	9.3	3.1

Stevens and Higgins state that the corn-picking season in Maryland has a much higher average temperature than the corresponding season in Maine, the difference being sufficient to cause considerably greater deterioration in picked corn during a given period.[1] They also derived the exponential and physiological indices corresponding to the daily normal temperatures for the corn-canning seasons of both localities. The means of these two sets of indices were both greater for Baltimore, Md., than for Portland, Me.; but they were unable to decide which method furnishes the best criteria of the relative rates of deterioration of picked corn in the two localities. The data presented in this paper and in a previous paper by Appleman and Arthur[2] lend support to the exponential indices as a good measure of the relative climatic temperature efficiency for the deterioration of picked corn in different localities.

The quality of canned corn may be influenced not only by the temperature at which the corn is handled but also by the effect of temperature on the rate of ripening. A slow rate of ripening gives a greater range in the number of days that the corn may be picked in good condition. Corn that ripens in very warm seasons, for example in the month of August in Maryland, requires very close attention lest the best stage for picking be allowed to pass. The data presented in this paper should furnish a more rational basis for picking green sweetcorn.

SUMMARY

Sweetcorn is considered ripe when the growth of the kernels ceases and the chemical changes in the corn have nearly attained equilibrium positions. The maturing of corn consists essentially in the loss of water.

The chief changes in percentage composition of corn during ripening consists in the depletion of sugars and the increase of starch.

In the very early stages of ripening the reducing sugars predominate; therefore the stage of highest total sugar content does not necessarily coincide with the stage of greatest sweetness.

[1] STEVENS, Neil E., and HIGGINS, C. H. OP. CIT.

[2] APPLEMAN, Charles O., and ARTHUR, John M. OP. CIT.

Calculated as percentages of dry weight, the changes in fat, crude fiber, and total nitrogen occur during the very early stages of ripening. For the remainder of the ripening period these percentages remain fairly constant.

The rate of starch synthesis in the kernels seems to be the controlling factor for several supplementary processes. The rate at which the ratio of total sugar to starch decreases is a good measure of the ripening rate and was employed for that purpose.

Temperature is the controlling factor for the rate of ripening in sweetcorn. Several temperature indices were employed to evaluate climatic temperature efficiency for the ripening processes. The exponential indices were found to furnish the best criteria of the temperature efficiency for sweetcorn ripening.

A late crop of corn required 15 days for the same period of ripening that required only 6 days for an early crop, a time ratio of 2.5. The averages of the daily exponential indices for the two seasons were practically in the same ratio. Therefore, the rate of ripening in sweetcorn, within a wide range of temperature, appears to adhere rather strictly to the Van't Hoff-Arrhenius principle.

The rate of ripening being inversely proportional to the exponential indices, a basis was furnished for an approximate prediction of the number of days required in different localities and at different seasons in the same locality for corn to pass from the beginning of kernel formation to the best edible stage, as well as the maximum number of days that the corn may be expected to remain in this condition.

SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM

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INTRODUCTION

The purpose of the present paper is to define the characters which will distinguish the larva and pupa of the pink bollworm, *Pectinophora gossypiella* Saunders, from those of other Lepidoptera attacking cotton or related malvaceous plants and of still others feeding on plants other than malvaceous but frequently found in the neighborhood of cotton fields. A few (*Dicymolomia julianalis* Walker and *Crocidosema plebeiana* Zeller, for example) so closely resemble the pink bollworm in their habits and their larval stages that they are only to be distinguished by a careful examination of their structure. It is hoped that the present paper will make the differentiating characters clear and will enable entomological workers to distinguish the forms treated.

The field work upon which this paper is based was conducted throughout the area in southeastern Texas where the pink bollworm has been found to occur, as well as in Cameron County, at the southern extremity of the State. Special attention was devoted to discovering whether the pink bollworm was attacking plants other than cotton. Thousands of seed pods of okra and other malvaceous plants were examined. In one case, at Smiths Point, in Chambers County, all the seed pods of a plant related to cotton (*Hibiscus lasiocarpus*), growing in the immediate vicinity of a field where a heavy infestation by the pink bollworm had occurred during a previous year, were removed and given minute examination. Similar investigations were made with reference to other wild and cultivated malvaceous plants growing in or about fields where the

[1] This study was conceived and arranged by Dr. W. D. Hunter, in charge of the Pink Bollworm Eradication, to aid the work of his inspectors. To the necessary preliminary field work the following entomologists were detailed by Dr. Hunter: H. C. Hanson, J. D. More, E. L. Diven, A. C. Johnson, and Carl Heinrich. For a short period Mr. Herbert Barber was also associated with the work. The material and notes on which the paper is based are all due to these workers. Especial mention should be made of Emerson Liscum Diven, who had a major part in the investigations and who lost his life in an aeroplane accident while scouting for cotton areas and who, had he lived, would have worked up the results as here given.

With the exception of Plate 107, all the drawings accompanying this paper were made under the writer's supervision by Mr. H. B. Bradford, of the Bureau of Entomology. Plate 107 (also originally by Mr. Bradford) is reproduced from Busck's article on the pink bollworm (*In* Jour. Agr. Research, vol. 9, no. 10, p. 343–370, 1917). The writer is especially indebted to Mr. Bradford for his painstaking and accurate drawings.

To Mr. Busck the writer is indebted for many helpful suggestions and both to him and to Dr. Dyar for verification of some of the identifications.

pink bollworm had been found. In no instance was the pink bollworm found in any plant other than cotton.

Thirty-eight species are considered here. Of these, six are described as new, and four, already described, are recorded for the first time from the United States. In each case the male genitalia of the type specimen of the new species are figured. The essential larval and pupal characters are referred to in the text as fully as possible, and purely descriptive matter is reduced to a minimum.

FAMILY GELECHIIDAE

PECTINOPHORA GOSSYPIELLA (SAUNDERS), THE PINK BOLLWORM

(PL. 101, A, B; 103, A; 105, C, E; 106, A; 107, A–D)

Depressaria gossypiella Saunders, 1843, *in* Trans. Ent. Soc. London, v. 3, pt. 4, p. 284–285.

Pectinophora gossypiella Busck, 1917, *in* Jour. Agr. Research, v. 9, no. 10, p. 343–370.

Inasmuch as the immature stages of the pink bollworm have been already fully described in an earlier number of this journal [1] it will be necessary here only to point out the structural characters which will serve to identify its larva and pupa and distinguish them from those of other Lepidoptera which, because of their habits, food plants, or general appearance, might be mistaken for *Pectinophora gossypiella*. There is no easy and ready-made method which will enable a layman to distinguish an insect and be certain of its identity. This applies with particular force to the pink bollworm. As Busck well states—

> Definite and final determination of *P. gossypiella* in any stage can be made only by the aid of a microscope

and he might have added, only by one reasonably experienced in insect determination and familiar with the characters used in classifying Lepidoptera. Nevertheless the pink bollworm has structural characters by which it can be determined and its identity established beyond the possibility of doubt. The specialist alone can pass upon these with certainty; but the average intelligent worker in the field can also use them, far enough at least to say what larvæ or pupæ commonly found in and about cotton fields can not be *P. gossypiella*.

The combination of the following characters distinguishes the larvæ of the pink bollworm:

Three setæ (III, IV, and V) triangularly grouped on the prespiracular shield of the prothorax (T1). (Pl. 103, A.)

Setæ IV and V closely approximate on the proleg-bearing abdominal segments (AIII). (Pl. 103, A.)

Setæ III above (not directly before) the spiracle on the eighth abdominal segment (AVIII).

[1] BUSCK, August. THE PINK BOLLWORM, PECTINOPHORA GOSSYPIELLA. *In* Jour. Agr. Research, v. 9, no. 10, p. 343–370, 7 fig., pl. 7–12. 1917. Literature cited, p. 366–370.

On the ninth abdominal segment (Aix) the paired dorsal setæ II not on a single pinaculum (chitinized plate) and not appreciably closer together than the paired I on the dorsum of the eighth abdominal segment; seta I no nearer to III than to II; VI closely approximate to IV and V; group VII unisetose.

Prothoracic legs appreciably separated at their base. No anal fork on tenth abdominal segment. Crochets of abdominal prolegs uniordinal and arranged in a circle broken outwardly. (Pl. 106, A.)

On each side of the thoracic shield near Seta I^b a small crescent or reniform spot (Pl. 103, A) paler than the surrounding chitinized area.

On the epicranium the lateral seta (L^1) behind the level of P^1 and *remote* from A^3 (that is, farther from A^3 than A^3 is from A^2) and the anterior puncture (A^a) *lying between* setæ A^1 and A^2. (Pl. 101, A.)

Each of these characters is possessed by other lepidopterous larvæ, but their combination is peculiar to *Pectinophora gossypiella*. No other known larva that we have in this country possesses them all. I have not seen caterpillars of (*Gelechia*) *Pectinophora malvella* Zeller,[1] the only other known species of the genus Pectinophora, or of *Platyedra vilella* Zeller, which Meyrick considers congeneric with *Pectinophora gossypiella*.[2] These may have most or all of the structural characters here given, but as neither of them occurs outside of the Old World they do not concern us at present.

The setal characters are fully illustrated on Plates 101, 103, and 105. It will be noted that two slight changes have been made from the drawings published in Busck's paper. The numbering of abdominal setæ IV and V has been reversed to correspond with our present conception of the homologies of these setæ; and the lateral puncture (L^a) of the epicranium is shown directly posterior to rather than postero-ventrad of seta L^1. In Busck's figures[3] the puncture is much too low.

The pupa (Pl. 107, A–D) is evenly and densely clothed with a fine pubescence; moderately stout, with a short, hooked cremaster surrounded by 6 to 8 stout, hooked setæ but *without* dorsal spines or other armature; labial palpi absent; maxillary palpi long, extending four-fifths of the wing length; antennæ long but not quite reaching to tips of wings; vertex distinct but narrower than prothorax.

No other lepidopteron feeding on malvaceous plants in this country has such a pupa. The fine pubescence and short, hooked cremaster are easily discernible under a small hand lens and are enough to identify the pupa which, when once seen, is not likely to be confused with that of any other cotton-feeding species.

[1] After this paper had gone to the printer we received from the Abbé J. de Joannis of Paris a larva of *Pectinophora malvella*. The structural characters are the same as those of *Pectinophora gossypiella*.

[2] The Abbé Joannis also sent us a male moth of *Platyedra vilella*. A comparison of the genitalia of this and *Pectinophora gossypiella* does not support Meyrick's contention.

[3] Busck, August. op. cit., 1917, p. 348, fig. 2, B.

GELECHIA HIBISCELLA BUSCK

(PL. 93, C)

Gelechia hibiscella Busck, 1903, *in* Proc. U. S. Nat. Mus., v. 25, p. 869–871.
Gelechia hibiscella Busck, 1903, *in* Dyar, List North Amer. Lep., no. 5739.

This species was originally described from larvæ collected on *Hibiscus moscheutos* in the vicinity of Washington, D. C.

On the shores of Miller's Lake and Lake Charlotte in Chambers Co., Tex., we found the larvæ fairly abundant in early September (1918) on both *Hibiscus lasiocarpus* and *H. militaris* and also occasionally on *Kostelezkya* spp. During October of the same year adults were reared from these. The male genitalia compared with those of typical specimens from the type locality agree in all details. A figure of the elaborate and characteristic genitalia is given in Plate 93, C.

Gelechia hibiscella seems to be limited in food plant to Hibiscus and one or two other closely allied Malvaceae. We have never found it on cotton or okra, but there seems to be no reason why it should not thrive on these. The feeding habits vary somewhat according to the characters of the plant on which the larvæ feed. On the broader-leaved *Hibiscus moscheutos* around Washington and the similar *H. lasiocarpus* in Texas the larvæ feed chiefly on the leaves, rolling them up and partially biting through the stems before pupation so that the folded leaf is easily shaken to the ground by a slight wind. Within this roll they pupate. Occasionally the larvæ also attack the seed pods, but from the writer's observation this is rather rare in the broad-leaved species of Hibiscus. In the narrow-leaved *H. militaris* and in *Kostelezkya* spp., on the other hand, the habits are quite different. Here the larvæ feed chiefly in the flowers and seed pods, pupating in the withered flowers, and do not attack or use the leaves at all.

There is no possibility of confusing this species with *Pectinophora gossypiella*. The larvæ as well as adults of the two are very different. In *Gelechia hibiscella* the body of the larva from the beginning of the metathoracic segment to the caudal end is white, longitudinally marked with continuous, narrow, somewhat wavy, reddish brown stripes; one pair on the dorsum, lying between the paired setæ I; one subdorsal stripe on each side, above seta III, and a lateral stripe in the spiracular area. Except on the metathoracic and ninth abdominal segments none of the body tubercles are touched by the longitudinal stripes but lie between them on the white areas. The first two thoracic segments are reddish brown with the anterior portion of the mesothorax white above. The anal shield is yellow; the thoracic legs and prothoracic shield are black. The chitinizations about body tubercles moderate but conspicuous, black or blackish brown, rounded or oval, and sharply defined; crochets of prolegs uneven biordinal and in a complete circle, 32 to 36, brown; anal fork present, rather stout, 6- to 8-pronged; head yellow-brown,

more or less suffused and mottled with black; ocellar pigment black, continuous under all the ocelli. Full-grown larvæ 22 to 23 mm. long.

The only caterpillar treated in this paper which could easily be confused with this species is that of *Gelechia neotrophella* Heinrich. The latter, however, is at once distinguished by its 2-pronged anal fork and the fusing of the middorsal stripes on most of the abdominal segments.

GELECHIA BOSQUELLA CHAMBERS

Gelechia bosquella Chambers, 1878, *in* Bull. U. S. Geol. Surv. Terr., v. 4, p. 87.
Gelechia basquella Busck, 1903, *in* Dyar, List North Amer. Lep., no. 5729.

A single moth of this species was reared September 23, 1918, from *Cassia tora* infested by larvæ of *Platynota rostrana* Walker, collected at Turtle Bayou, Tex. This species is not a malvaceous feeder and is only mentioned here on account of the similarity of its larva to those of two other species treated in this paper, *Borkhausenia diveni* Heinrich and *Noctuelia rufofascialis* Stephens. It is very strikingly colored, the three thoracic segments being a bright wine-red while the rest of the body is green. The head, legs, thoracic shield, and body tubercles are black. The red coloring of the thoracic segments, however, is not continuous as in the two species just mentioned but is broken on the anterior portion of the meathtorax by a broad encircling band of the greenish body color.

A detailed technical description of the larva is given by Dyar in Busck's revision of the American Gelechiidae.[1]

GELECHIA NEOTROPHELLA, N. SP.

(PL. 94, C-G; 105, H)

Gelechia neotrophella, n. sp.

Antennæ black. Palpi black, dusted with white. Face black, very slightly dusted with white. Head and thorax black, heavily dusted with white. Forewings black, marked with overlaid white scales; the white dustings over the black forming an oblique, basal grayish-white patch wider on dorsum than on costa, an obscure, rather broad median fascia consisting of a narrow, oblique median streak clouded with grayish before and behind, and a short white geminate costal dash at apical fourth; cilia smoky blackish fuscous. Hindwings and cilia pale smoky fuscous, somewhat shaded with black toward apex. Legs black, dusted and annulated with white. Male genitalia of type as figured (Pl. 94, C–G). Alar expanse 12 to 13 mm.

HABITAT.—Brownsville, Tex. (Diven and Heinrich).

FOOD PLANT.—*Mimosa berlandieri*. Larva a leaf-tier, spinning a tube of silk as it feeds and so binding the leaves together.

TYPE.—Cat. No. 23739, United States National Museum.

Described from one male type and two male and six female paratypes. Two generations were noted. From larvæ collected February 3, 1919, moths issued March 5, and from larvæ put in rearing early in May, 1919, adults emerged toward the end of the same month.

[1] BUSCK, August. A REVISION OF THE AMERICAN MOTHS OF THE FAMILY GELECHIIDAE, WITH DESCRIPTIONS OF NEW SPECIES. *In* Proc. U. S. Nat. Mus., v. 25, no. 1304, p. 864–865. 1903.

The larva is yellowish white, longitudinally striped with wine-red; one rather broad middorsal stripe dividing into two thin parallel stripes from the second abdominal segment forward; one moderately broad subdorsal and one lateral stripe extending from hind margin of prothorax and fusing on the ninth abdominal segment and forming on the tenth a dark border around the outer edge of the anal shield; in the area of seta VI a similar narrow sublateral stripe; head and thoracic shield pale yellow; crochets of prolegs 28 to 34, biordinal and arranged in a complete circle; anal prolegs with a conspicuous blackish red chitinized spot on caudal side; anal fork rather large, 2-pronged; full-grown larva 8 to 8.5 mm. long.

The species is close to and strikingly resembles *Gelechia trophella* Busck, from which, however, it is easily distinguished by the male genitalia. The structural differences are shown in Plate 93, A and B, and in Plate 94, C–G.

The larva is not in any way to be confused with the pink bollworm, from which it differs strikingly in superficial appearance. It resembles somewhat the larva of *Gelechia hibiscella* Busck but is separable from that species by food plant and structure. In *G. neotrophella* the anal fork is 2-pronged, while in *G. hibiscella* it has from 6 to 8 distinct prongs. In the latter, also, the dorsal stripes are nowhere fused.

TELPHUSA MARIONA, N. SP.

(PL. 94, A, B; 105, F; 109, G)

Telphusa mariona, n. sp.

Antennæ black. Palpi cream-color, shading to white on upper side of second joint; apical half of third joint and upper side of basal joint black. Face white. Head and thorax cream-yellow. Forewings glossy black with two conspicuous cream-colored spots; one, a short triangular dash on outer third of costa; the other, an irregular spot of about the same size on dorsum just beyond middle; in some specimens two or three minute and obscure patches of white or cream-colored scales along termen; cilia blackish. Hindwings and cilia smoky fuscous. Legs black, ringed at outer margins of the joints with cream-yellow or white. Male genitalia of type as figured (Pl. 94, A, B). Alar expanse 9 to 11 mm.

HABITAT.—Brownsville, Tex. (J. D. More and H. C. Hanson).

FOOD PLANT.—*Abutilon incanum*. Larva a leaf-folder. Also taken on *Abutilon berlandieri*, *Malvastrum* sp., *Wissadula* sp., and *Sida* sp.

TYPE.—Cat. No. 23740, United States National Museum.

Described from male type and 25 male and female paratypes reared from larvæ collected in late March and early April, 1919, on *Abutilon incanum*. Moths issued from middle of April to middle of May, 1919.

Larva, full-grown, 6.5 to 7 mm. long; slender. Body yellowish white with a subdorsal and a lateral longitudinal row of large red blotches and a longitudinal row of smaller red spots on the level of seta VI and just anterior to that seta on each segment; on the eighth abdominal segment the paired subdorsal spots are fused and on abdominal segment 9 the

subdorsal and lateral spots are also fused; legs pale yellow; crochets light brown, 18 to 20 in a complete circle, unevenly biordinal; thoracic shield divided by a thin median longitudinal pale line, yellow with a broad shading of fuscous on the lateral extremities and a smaller fuscous patch at the center of the anterior dorsal margin; anal shield yellow laterally shaded with fuscous; other chitinized areas smoky fuscous, tubercles moderately chitinized; hairs moderately long, slender, yellowish. Head light yellow with a narrow black shading at posterior lateral incision of hind margin and a similar black dash on ventral margin of epicranium adjacent to triangular plate of hypostoma; ocellar pigment black, continuous under all the ocelli.

The larva is very similar in superficial appearance to the scavenger worm (*Pyroderces rileyi* Wlsm.). It differs most strikingly in the arrangement of the red markings, which are in spots or blotches rather than in continuous bands, and in the possession of a well-developed anal fork (Pl. 105, F) entirely lacking in *P. rileyi* and the pink bollworm.

The pupa is easily distinguished from those of the other Lepidoptera treated in this paper by the peculiarly scalloped and fringed posterior margin of its eighth abdominal segment. (Pl. 109, G.)

ISOPHRICTIS SIMILIELLA (CHAMBERS) [1]

(PL. 95, A; 102, F)

Gelechia similiella Chambers, 1872, *in* Canad. Ent., v. 4, p. 193.
Paltodora similiella Busck, 1903, *in* Dyar, List North Amer. Lep., no. 5548.

In the dead flower heads of *Rudbeckia* sp. (commonly called "nigger heads" in many parts of Texas) there are two species of lepidopterous larvæ which many nonentomologists have confused with *Pectinophora gossypiella*. One of these when mature is about the same size as and superficially like a full-grown pink bollworm. It is an olethreutid, however, and as such is easily distinguished by the setal arrangement of the ninth segment which readily separates the two families Gelechiidae and Olethreutidae. In the former the paired setæ II on the dorsum of the ninth segment are no closer together than the paired setæ I on the dorsum of abdominal segment 8 (Pl. 105, C) and I is as near II as it is III on the ninth abdominal segment. In the Olethreutidae, on the other hand, the paired II on the dorsum of the ninth abdominal segment are on a single chitinization and *closer together* than the paired I on the eighth abdominal segment. Also I and III are closely approximate (Pl. 105, B). We have not succeeded in rearing the moth, so specific determination can not be given. The family position of the larva, however, is certain.

[1] The genus Isophrictis has been erected by Meyrick for those species formerly listed under the genus Paltodora Meyrick having the second joint of the labial palpi clothed beneath with long rough spreading hairs and having veins 7 and 8 of forewings out of 6. It replaces Paltodora for the North American species. (MEYRICK, E. ON THE GENUS PALTODORA. *In* Ent. Mo. Mag., v. 53, no. 636 [s. 3, v. 3, no. 29], p. 113. 1917.)

The other Rudbeckia feeder (*Isophrictis similiella* Chambers) belongs to the same family as the pink bollworm and is much more abundant and less local than the olethreutid. It feeds on the seeds of a number of Compositae and is frequently found in sunflower heads. The larva when mature often has a pinkish tinge and somewhat resembles an immature pink bollworm except for its shape, which is distinctly spindle-like, sharply tapering at both ends and decidedly stout for its length (1.5 to 2 mm. wide by 5 mm. long in full-grown specimens). The arrangement of the setæ of the anterior group on the epicranium is also characteristic; A^1, A^2, and A^3 are crowded very close together on the anterior dorsal part of the head and L^1, while remote from A^3 as in most Gelechiidae, is well forward near the ocelli. (Pl. 102, F.)

The pupa shows under the microscope a slight pubescence similar to that of *Pectinophora gossypiella* but this is *limited to the head alone.* Otherwise, except for the normal setæ and a sharp, thorn-like, *dorsally projecting* cremaster, the pupa is smooth. It is short and moderately stout (1.5 mm. broad by 5.5 to 6 mm. long) with the wing cases reaching nearly to and the metathoracic legs extending a trifle beyond the tip of the abdomen.

Several moths of this species were reared from larvæ collected at various points in Chambers County and in the neighborhood of Galveston and Houston. Larvæ were collected in late August and early September, 1918, and adults issued from these from the middle to the end of September the same year. Other larvæ, taken in October of 1918, produced moths the following May, passing the winter as pupæ within the dried flower heads.

The male genitalia of the moth are figured in Plate 95, A.

FAMILY OECOPHORIDAE

BORKHAUSENIA DIVENI, N. SP.

(PL. 96, C–F)

Borkhausenia diveni, n. sp.

Antennæ white, faintly annulated with fuscous above. Palpi blackish fuscous, broadly banded at base and apex of third joint with white; inner sides somewhat dusted with white scales. Face white. Head white with a slight suffusion of fuscous at vertex. Thorax white, heavily dusted with blackish fuscous; tegulæ white, basal half blackish fuscous. Forewings white, suffused and mottled with pale brown and black scales, the brown suffusion obscuring most of the ground color at the base and beyond the middle of the wing; an irregular black spot at base of costa; a similar black spot on lower vein of cell close to base; above and below it two smaller black spots; at middle of wing a straight, rather broad, vertical fascia of blackish brown scales inwardly margined by a distinct line of the white ground color; in the middle of this fascia a round spot of distinctly paler brown scales with the black scales edging it slightly raised; on costa just beyond median fascia a poorly defined triangular patch of brown and blackish scales; a small black dot at upper outer angle of cell and several small obscure dark spots near tornus; cilia dirty white. Hindwings and cilia grayish

fuscous. Legs fuscous on outer sides; banded with white on middle of tibiæ and at ends of joints; white on inner sides. Male genitalia of type figured (Pl. 96, C–F). Alar expanse 12 to 13 mm.

HABITAT.—Brownsville, Tex. (E. L. Diven).

FOOD PLANT.—*Lantana horrida*. "Larvæ making a narrow blotch mine at the edge of the leaf and curling the edge near base, pupating within the mine" (Diven note).

TYPE.—Cat. No. 23741, United States National Museum.

Described from male type and one male and three female paratypes reared from larvæ collected April 22, 1919. Moths issued April 27 to May 5, 1919. Named in honor of the late Emerson Liscum Diven.

The larva when full-grown is 7.5 to 9 mm. long; white, with the thoracic segments and the anterior portion of the first abdominal segment a brilliant wine-red; in fully fed specimens there is often a pinkish suffusion on the dorsum of the abdominal segments; thoracic shield yellow, posteriorly and laterally edged with dark brown; anal shield pale yellow; other chitinized portions of thoracic segments dark brown; thoracic legs blackish brown, paler on inner sides; body tubercles deep brown, minute; setæ pale, slender, moderately long; crochets of prolegs dark brown, 24 to 26, biordinal and in a circle broken outwardly; spiracles pale yellow, small, round, inconspicuous; no anal fork; head pale yellow with a dark brown band on each side, extending from the ocelli to the lateral incision of the hind margin; ocellar pigment black, continuous under the ocelli.

The pupa is rather stout and short, 1.5 to 2 mm. wide by 4.5 to 5 mm. long; pale yellow-brown; smooth; caudal end rounded; cremaster absent; wings and antennæ extending to anterior margin of sixth abdominal segment; labial palpi clearly defined but small, *not* extending to proximolateral angles of maxillæ; between genital and anal openings a divided, blackish, chitinized rise, without spines, hairs, or other armature.

This species is easily distinguished from the other American forms in the genus by the straight median fascia. I have placed it in Borkhausenia advisedly, although strictly speaking it does not belong there. In any further revision of the Oecophoridae, *Borkhausenia divini* with *B. conia* Wlsm., *B. fasciata* Wlsm., *B. episcia* Wlsm., and probably *B. orites* Wlsm., will have to be placed in a new genus. While agreeing with the type of Borkhausenia (*B. minutella* L.) on venational characters, they differ markedly in genitalia. In *B. minutella* (Pl. 96, A, B) the harpes are typically oecophorid and laterally placed, the uncus present though small, the eighth abdominal segment simple, and the entire apparatus symmetrical. In *B. diveni* and its allies, on the other hand (Pl. 95–97), the eighth abdominal segment is distinctly modified, the uncus is absent, the harpes more ventrally placed, and the genital apparatus consistently asymmetrical. The characters of their genitalia are those of the genus Triclonella Busck, from which the species are separable on venation, *B. diveni* and its allies having 5 of the hind wing distinctly separate at base from the stalk of 3 and 4. The presence of a few raised scales on

the forewing would seem to throw *B. diveni* into Meyrick's genus Erysiptila. The latter, however, is again distinct on characters of genitalia on which it will have to be retained and recharacterized, as the raised scale character does not seem to hold. It is possessed by *B. diveni* but not by the other closely allied species (*B. conia*, *B. fasciata*, etc.). The genus Erysiptila, while similar to these in some genitalic characters (for example, the peculiar development of fused and armed soci and gnathus) and thus separable from the genus Borkhausenia, has the organs symmetrical throughout and the harpes laterally rather than ventrally placed. Of the North American species now listed under the genus Borkhausenia only three (*B. pseudospretella* Staint., *B. haydenella* Chambers, and *B. ascriptella* Busck) agree with the type species on all characters. For the present, however, *B. diveni* and its allies may be retained in that genus. Until the entire family can be revised along lines suggested by the development of genitalic structures there is nothing to be gained by erecting a single genus on these characters.

FAMILY STENOMIDAE

AEDEMOSES HESSITANS WALSINGHAM[1]

(PL. 95, B, C.; 104, D)

Aedemoses hæsitans Walsingham, 1912, *in* Biol. Centr.-Amer., Lep. Heter., v. 4, p. 154.

Eighteen specimens (males and females) of a moth which Mr. Busck has determined as this species were reared by Diven from larvæ which he had collected on "Mexican ebony" (*Siderocarpus flexicaulis*) at Brownsville, Tex. The genus and species were described by Walsingham from a unique female without hind legs, collected at Presidio Durango, Mexico, and have not since been recorded. The present rearing, therefore, adds another to our list of United States species. There can be no doubt of the identification, as Busck has seen and is familiar with the Walsingham type and our reared examples agree in all details with the description.

The larva is a leaf-tier, binding together several leaves and feeding within the tie, eating first the epidermis and later all but the veins of the leaves. It pupates within the tie, the pupa being naked and attached at its caudal end by a strand of silk to one of the leaves.

The larva is a typical stenomid, slightly flattened and with seta III *antero-dorsad* of and close to the spiracle on abdominal segments 1 to 7 (Pl. 104, D); body white with four pale purplish brown longitudinal stripes, one subdorsal pair just below the level of setæ I and II, and a dorso-lateral one just above the level of setæ III; thoracic and anal shields pale yellow; thoracic legs pale yellow, lightly shaded with brown;

[1] Meyrick sunk the genus Aedemoses Walsingham as a synonym of the genus Stenoma Zeller, but on insufficient grounds, as he disregards its very distinct venational structure in favor of general appearances. (MEYRICK, E. EXOTIC MICROLEPIDOPTERA, v. 1, pt. 13, p. 412. 1915.)

body tubercles inconspicuous, chitinized areas about them unpigmented except around setæ II[a] and II[b] on mesothorax and metathorax where they are pale brown; body hairs whitish yellow, rather long; abdominal crochets yellow, 40 to 44, unevenly biordinal and in a complete circle; anal fork absent; head pale yellow, the more heavily chitinized parts of trophi lighter brown; ocellar pigment black, continuous under the ocelli; length, full grown, 7 to 7.5 mm.

The pupa is the typical short, squatty stenomid form; smooth, without armature or processes of any kind except the very short, inconspicuous primary setæ and a pair of minute spines on the anal rise; seta III on abdominal segments well forward of the spiracle; spiracles distinct and rather large, very slightly produced; wings, antennæ, and metathoracic legs extending to anterior margin of fifth abdominal segment; antero-ventral margins of fifth abdominal segment curved around the edge of the wing tips; labial palpi very small, not reaching to proximo-lateral angles of maxillæ; eighth, ninth, and tenth abdominal segments considerably reduced and sharply tapering; prothorax broad, nearly one-third the breadth of mesothorax; proleg scars distinct; length 4 to 4.5 mm; width 1.5 to 2 mm.

Immature larvæ were collected by Diven in late January, 1919, and feeding larvæ as late as April 1, 1919; from the latter, moths issued from April 17 to 26 of the same year.

The male genitalia of the moth are figured in Plate 95, B, C.

FAMILY BLASTOBASIDAE

ZENODOCHIUM CITRICOLELLA (CHAMBERS)

(PL. 98, A–C; 102; 104, C; 105, I)

Blastobasis citricolella Chambers, 1880, *in* Rept. U. S. Dept. Agr. 1879, p. 206–207.

Blastobasis citriella Chambers, 1880, *in* Rept. U. S. Dept. Agr. 1879, p. 245.

Zenodochium citricolella Dietz, 1910, *in* Trans. Amer. Ent. Soc., v. 36, p. 11–12.

Feeding in dry okra pods, in the seed pods of Hibiscus, and in old or diseased cotton bolls we often found associated with *Pyroderces rileyi* a dirty brownish larva with a glistening black head and thorax, spinning a thin web in the seed pods within which it fed and pupated. A number were collected at various places in Chambers County (Smith Point, Point Bolivar, and South Bayou) and from these were reared a number of adults agreeing in genitalic and other characters with authentic reared specimens of *Zenodochium citricolella* Chambers in the United States National Museum. The species is a scavenger and probably a very general feeder, as it was originally recorded from dried oranges and is to be found in almost any dry or diseased malvaceous seed pod.

Figures of the male genitalia of the moth are given in Plate 98, A–C.

The larva is easily distinguished from *Pyroderces rileyi* and the other lepidopterous cotton feeders by the structural characters shown on Plates 102, 104, and 105. The most striking features are the oval chitinized plate on the submentum, the nearly complete fuscous circle surrounding the chitinization of tubercle III on abdominal segments 1 to 7, and the typical blastobasid arrangement of the prothoracic legs (Pl. 105, I), set very close together with the coxal lobes touching each other.

The species probably has several generations a year. Larvæ collected in August, 1918, produced moths in that month and throughout September. Others collected during November and December produced moths the following April.

HOLCOCERA OCHROCEPHALA DIETZ

(PL. 98, D–F)

Holcocera ochrocephala Dietz, 1910, *in* Trans. Amer. Entomol. Soc., v. 36, p. 31–32.

A large series of moths were reared during February and March, 1919, from larvæ collected December, 1918, in imperfectly opened and weevil-infested cotton bolls at Brownsville, Tex. They agree with the description and the single female paratype of Dietz's species in the United States National Museum, and I have no hesitation in so determining them. The larval habits are the same as those of *Zenodochium citricolella*. There probably has been some confusing of our material, as all the larvæ we have associated with the *H. ochrocephala* adults are identical with those of *Z. citricolella*. Probably, since the two species work together in the same way and are superficially alike, the larvæ of one species was preserved and that of the other reared. It is extremely unlikely that there should be two blastobasids in different genera without a single structural difference in their larvæ.

The male genitalia of the moth are figured in Plate 98, D–F.

HOLCOCERA CONFAMULELLA, N. SP.

(PL. 99, C)

Holcocera confamulella, n. sp.

Antennæ deeply excised above basal joint and with truncate scale tuft; very weakly ciliate. Palpi grayish ochreous, dusted with fuscous on outer sides. Face grayish ochreous, vertically banded with fuscous. Head and thorax grayish white mixed and suffused with fuscous scales. Forewings grayish white, suffused and mottled with fuscous, the fuscous scaling giving the outer two-thirds of the wing a distinctly gray-brown appearance, darkening into an ill-defined, outwardly angulate antemedial fascia bordering a grayish basal patch and forming an irregular, broken, and obscure vertical fascia beyond the middle; along the termen a few barely distinguishable fuscous spots; cilia grayish white. Hindwings very narrow, pale smoky fuscous; cilia paler, tinged with ochreous. Legs whitish ochreous on inner sides; the outer sides fuscous, spotted with white on tibiæ and ringed with white or whitish ochreous at ends of joints. Male genitalia of type figured (Pl. 99, C). Alar expanse 14 to 15 mm.

HABITAT.—Brownsville, Tex. (More, Barber, Heinrich).
FOOD PLANT.—Fruits of Crataegus.
TYPE.—Cat. No. 23742, United States National Museum.

This species is very close to *Holcocera modestella* Clemens, to which it would run in Dietz's tables.[1] It may eventually prove to be that species, but in the absence of an authentic male of *H. modestella* from the type locality it is better to risk a possible synonym than to make a doubtful determination. I have seen no specimens of Clemens's species. The male genitalia here figured fix the concept of *H. confamulella* and enable its ready identification.

Five moths (male type and four male and female paratypes) were reared April 10 to 21, 1919, from fruits of Crataegus rather heavily infested by larvæ of *Crocidosema plebeiana* Zeller. The larvæ of *Holcocera confamulella* were not noted.

FAMILY ETHMIIDAE

ETHMIA DELLIELLA (FERNALD)

Psecadia delliella Fernald, 1891, *in* Canad. Ent., v. 23, p. 29.
Babaiaxa delliella Busck, 1903, *in* Dyar, List North Amer. Lep., no. 5935.
Ethmia delliella Barnes and McDunnough, 1917, Check List Lep. Bor. Amer., no. 6645.

One moth reared April 30, 1919, from *Wissadula lozani* heavily infested by a stem-boring aegeriid (*Zenodoxus palmi* Neumoegen). Material collected at Brownsville, Tex., by E. L. Diven, March 28, 1919. Larva and habits not noted.

ETHMIA BITTENELLA (BUSCK)

Tamarrha bittenella Busck, 1906, *in* Proc. U. S. Nat. Mus., v. 30, p. 730.
Ethmia bittenella Meyrick, 1914, Lep. Cat., pars. 19, p. 28.

Two pupæ collected by Diven in galleries in stems of *Wissadula lozani*, Brownsville, Tex., April 1, 1919. Moth issued April 9, 1919.

The larvæ were not noted. The caterpillars of this family are, however, to be distinguished from the others having three setæ on the prespiracular shield of prothorax and IV and V of abdomen approximate by the presence of one or more secondary hairs on the body, usually on the abdominal segments in the region of the prolegs. The prolegs themselves are long and slender as in the Pterophoridae. On abdominal segment 9, seta I is higher than II.

[1] DIETZ, WM. G. REVISION OF THE BLASTOBASIDAE OF NORTH AMERICA. *In* Trans. Amer. Ent. Soc., v. 36, no. 1, p. 24-33. 1910.

FAMILY COSMOPTERYGIDAE

PYRODERCES RILEYI (WALSINGHAM)

(PL. 102, A, B; 103, C; 105, D; 106, C; 107, E, F)

Batrachedra rileyi Walsingham, 1882, *in* Trans. Amer. Ent. Soc., v. 10, p. 198–199.
Batrachetra rileyi Dyar, 1903, List North Amer. Lep., no. 6059.
Pyroderces rileyi Busck, 1917, *in* Jour. Agr. Res., v. 9, no. 10, p. 362–366, 370.

The larva of this common scavenger is frequently mistaken for the pink bollworm. It is, however, very readily distinguished from it and similar pink-banded larvæ of the gelechioid and other groups.

Since a complete description of adult, larva, and pupa is given in Busck's article on the pink bollworm,[1] it will suffice here to call attention to the diagnostic characters of the immature stages.

For the larva these are:

Three setæ (III, IV, and V) triangularly grouped on prespiracular shield of prothorax; prothoracic II[a] higher than I[a]; IV and V on prolegbearing abdominal segments approximate; III on eighth abdominal segment *anterior to the spiracle;* paired dorsal setæ (II) on the ninth abdominal segment *not on a single chitinization,* but closer together than paired I on eighth abdominal segment (Pl. 105, D); *I and III approximate on ninth abdominal segment* (as in the Olethreutidae); IV and V approximate, with VI well separated from them on ninth abdominal segment; crochets of prolegs uniordinal and in a complete circle; anal fork absent; pink bandings on anterior and posterior margins (not in the middle) of the segments.

The sum total of these characters is possessed by no other caterpillar to be found on cotton.

The pupa (Pl. 107, E, F) may be distinguished by the following characters:

Pointed wing cases reaching to posterior margin of the sixth abdominal segment; antennæ reaching to tips of wings; maxillary palpi small and not reaching proximo-lateral angles of maxillae; vertex wider than prothorax; abdomen tapering, bluntly rounded, smooth except for primary hairs and a cluster of strong hooked setæ at posterior end and around anal opening; cremaster absent; no labial palpi or exposed metathoracic legs.

The drawings (Pl. 102, 103, 105–107) show the distinguishing structural characters of larva and pupa. It will be noted that a correction has been made in Busck's figure of the setal map of the ninth abdominal segment of the larva which omitted one of the ventral setæ. The setal arrangement of the ninth abdominal segment with all setæ in a row, I approximate to III and VI well-separated from IV and V, can not be

[1] BUSCK, August. OP. CIT. 1917, p. 362–366.

considered a family character. It serves, however, to separate *Pyroderces rileyi* from the gelechioid forms which it otherwise resembles.

FAMILY TORTRICIDAE

PLATYNOTA ROSTRANA (WALKER)

(Pl. 104, A; 105, A)

Teras rostrana Walker, 1863, *in* List Lep. Brit. Mus., pt. 28, p. 290.
Platynota rostrana Dyar, 1903, List North Amer. Lep., no. 5383.

This species and the following two are rather general feeders and are frequently found on cotton and other Malvaceae. We have reared moths of *Platynota rostrana* from cotton, okra (*Hibiscus esculentus*), *Malvaviscus drummondii*, *Bastardia viscosa*, *Amaranthus* spp., and *Cassia tora*, collected at Brownsville and several localities in Chambers County. The species is normally a leaf-feeder, tying the terminal leaves and pupating within the tie. We have, however, also found it occasionally feeding on the flower buds of okra and on one occasion (Dec. 31, 1918) Diven took three larvæ at Brownsville in dry cotton bolls, feeding on the lint. They pupated in the loose lint, and moths issued February 7 and March 3, 1919. In the Chambers County localities larvæ were collected during late August and early September, 1918, which produced moths late in September and early in October of the same year. There are at least two and probably three or more generations a year in Texas.

The larva is not likely to be confused with the pink bollworm. It is easily separable on the setal characters figured on Plates 104 and 105. The arrangement of the pared dorsal setæ (II) on the ninth abdominal segment (that is, *on a single chitinization* and considerably closer together than any dorsal pair on the eighth abdominal segment) (Pl. 105, A), coupled with the normal micro characters of three setæ on the prespiracular shield of prothorax, and a close approximation of IV and V on the proleg-bearing abdominal segments, distinguishes the families of the Tortricoidea. In Tortricidae proper (to which this and the two following species belong) seta I on the ninth abdominal segment is much as in the Gelechiidae (that is, rather well separated from III and often as near to II as to III) (Pl. 105, A). In the families Olethreutidae and Phaloniidae, on the other hand, I and III are approximate and very often on the same chitinization.

The pupa is typically tortricoid, with wings short and broad at the tip (not tapering) and having the abdominal segments armed dorsally with a double row of strong spines, those of the anterior rows larger and somewhat hooked (compare Pl. 108, D). It is distinguished from that of the common olethreutid malvaceous feeder (*Crocidosema plebeiana* Zell.) by the presence of a well-developed, bluntly rounded cremaster entirely lacking in the latter.

PLATYNOTA FLAVEDANA CLEMENS

Platynota flavedana Clemens, 1861, *in* Proc. Acad. Nat. Sci. Phila., 1860, p. 348.
Platynota flavedana Dyar, 1903, List North Amer. Lep., no. 5382.

One specimen reared by Diven (May 23, 1919) from cotton leaves collected at Brownsville, Tex., May 7, 1919.

The larva was not noted.

The pupa is strikingly like that of *Platynota rostrana* Walker.

AMORBIA EMIGRATELLA BUSCK

(PL. 109, F)

Amorbia emigratella Busck, 1910, *in* Proc. Ent. Soc. Washington, v. 11, p. 201–202.
Amorbia emigratella Walsingham, 1913, *in* Biol. Centr.-Amer., Lep. Heter., v. 4, p. 219.

Two moths reared from cotton May 19 and 24, 1919 (E. L. Diven) in same material infested by *Platynota flavedana*, collected at Brownsville, Tex., May 7, 1919. The pupa has a conspicuous mid-dorsal, cuplike, circular invagination near the anterior margins of the first seven abdominal segments, the anterior dorsal margins themselves being strongly chitinized and folded back into a projecting ridge; otherwise as in *P. rostrana*.

The larva was not noted.

FAMILY OLETHREUTIDAE

CROCIDOSEMA PLEBEIANA ZELLER

(PL. 99, A; 102, C, D; 103, E; 105, G; 106, B; 108, A–D)

Crocidosema plebeiana Zeller, 1847, *in* Isis von Oken, 1847, Heft 10, p. 721–722.
Eucosma plebeiana Walsingham, 1914, *in* Biol. Centr.-Amer., Lep. Heter., v. 4, p. 231–232.

Up to the present this almost cosmopolitan insect had not been recorded from the United States. Our collecting, however, showed it well distributed and fairly abundant in Texas. In the United States National Museum there are also several adults from California, so that its known range may be said to correspond roughly with the distribution of the Malvaceae. Adults were reared by us from the following plants: *Malvastrum spicatum* (Brownsville, Tex., May, 1919); hollyhock (*Althaea rosea*) (Brownsville, Tex., May, 1919); *Malvaviscus drummondii* (Smith Point, Tex., November, December, 1918; Anahuac, Tex., September, 1918); okra (*Hibiscus esculentus*) (Double Bayou, Tex., November, December, 1918); and *Kosteleyzkya* spp. (Anahuac, Tex., November, 1918). Larvæ were also collected in seed pods of *H. militaris* (Lake Charlotte, Tex., September, 1918) and in flowers of *H. rosa-sinensis* (Smith Point, Tex., November, 1918). They feed chiefly in the seed pods and on the seeds of

the plants infested, but occasionally also on the pollen of the flowers. The species is of special interest because its work and habits are almost identical with those of the genus Pectinophora and also because the larva is frequently pinkish and often has the outer crochets of the prolegs weakly developed or absent. It is easily mistaken for a half-grown pink bollworm. It is readily distinguished, however, by the structural characters here figured (Pl. 102, 103, 105, 106). The linear arrangement of setæ III, IV, and V on the prothorax, the position of III *anterior* to the spiracle on the eighth abdominal segment, the well-developed anal fork (Pl. 105, G), and the olethreutid grouping of the setæ on the ninth abdominal segment (Pl. 103, E) separate it from all the larvæ treated in his paper.

The characters of the pupal abdomen are shown on Plate 108, A–D. *Eucosma discretivana* Heinrich and *E. helianthana* Riley exhibit similar structures, but as neither of these species attacks Malvaceae there is little or no likelihood of confusing them with Crocidosema, We did not find *C. plebeiana* in cotton, but there appears to be no reason why it should not attack that plant; and its possible presence and confusion with the pink bollworm should be borne in mind in cotton inspection.

The male genitalia of the adult are shown in Plate 99, A.

EUCOSMA DISCRETIVANA, N. SP.

(PL. 99, B)

Eucosma discretivana, n. sp.

Antennæ, palpi, face, and head dull, somewhat ashy fuscous. Thorax pale, dull fuscous; tegulæ fuscous with a very slight bronzy tint. Forewings dirty grayish white marked with grayish fuscous; an outwardly angulate grayish fuscous basal patch slightly wider on costa than dorsum; a somewhat paler, semioval patch on dorsum before tornus and extending half way to costa; several narrow, obscure lines of fuscous scales extending outwardly from costa and faintly streaking the white areas; a similar faint line extending from dorsum through middle of white area bordering basal patch; entire termen narrowly margined by pale grayish fuscous: the whitish areas of the wing most pronounced just beyond basal patch and near tornus; cilia grayish; costal fold deeply appressed and reaching nearly to middle of wing. Hindwings dull, smoky fuscous, cilia grayish white with a dull fuscous band along their base. Abdomen grayish fuscous with silvery white scales along the sides and a few scattered silvery scales beneath. Legs fuscous, shading to dirty gray-white on inner sides. Male genitalia of type figured (Pl. 99, B). Alar expanse 13 to 16 mm.

HABITAT.—Sheldon, Tex. (A. C. Johnson).

FOOD PLANT.—"Wild myrtle." Larva boring in the stem and forming a gall.

TYPE.—Cat. No. 23743, United States National Museum.

Described from male type and three male and five female paratypes reared by A. C. Johnson, April 10 to 23, 1919, from larvæ collected by him March 14, 1919.

It is very close to *Eucosma obfuscana* Riley, which it strikingly resembles. The two species are, however, readily distinguishable on both genitalia and slight but constant color differences. In *E. obfuscana* the face, head, thorax, and base of antennæ are inky blue-black, the dark

margin of termen of forewing pronounced and blue-black, extending from the apex only a little over one-half the length of the termen, the white scaling of the tornal area extending into the cilia of the anal angle which are also white. In *E. discretivana* there is none of the blue-black scaling so noticeable in *E. obfuscana*, and the entire termen is faintly dark margined. The cucullus of the harpes of the male genitalia is also more narrowly elongate in *E. obfuscana* than in *E. discretivana*.

The larva is in general structure very like *Crocidosema plebeiana*, except that setæ I, III, IV, and V on the ninth abdominal segment are about equally spaced and the anal fork is lacking. The body is cream-white without markings; chitinized areas about body tubercles not pigmented; hairs whitish yellow; thoracic and anal shields pale yellow, scarcely pigmented; head light brown; crochets brown, 28 to 30, uniordinal and in a complete circle; length, full-grown, 10 to 10.5 mm.

The pupa is similar to that of *Crocidosema plebeiana* but somewhat larger, 8.5 to 9 mm. long by 2.5 mm. wide.

The two species are easily distinguished by their food plants and larval habits.

EUCOSMA HELIANTHANA (RILEY)

Semasia helianthana Riley, 1881, *in* Trans. St. Louis Acad. Sci., v. 4, p. 319.
Thiodia helianthana Dyar, 1903, *in* List North Amer., Lep., no. 5186.
Eucosma helianthana Barnes and McDunnough, 1917, Check List Lep. Bor. Amer., no. 7081.

We found a larva about the size of the pink bollworm and superficially resembling it feeding in the flower heads and on the seeds of the large garden sunflower. It was somewhat pinkish and had a pale kidney-shaped spot on the thoracic shield similar to that of Pectinophora. It had the characteristic olethreutid arrangement of setæ on the ninth abdominal segment and proved to be the caterpillar of *Eucosma helianthana* Riley, a species limited in food plant as far as I know to Helianthus. As the pink bollworm does not attack sunflower and *E. helianthana* does not attack cotton, there is no reason to confuse the two. The structural differences are also easily seen under a binocular or a strong hand lens.

The pupa is similar to that of *Crocidosema plebeiana* but larger, about the size of that of *Eucosma discretivana*.

Larvæ were collected at Dickinson, Tex., September 28, 1918, and pupæ also were found at Smith Point, August 30, 1918. From the latter a moth was reared September 3 of the same year.

LASPEYRESIA TRISTRIGANA (CLEMENS)

Stigmonota tristrigana Clemens, 1865, *in* Proc. Ent. Soc. Phila., v. 5, p. 133.
Enarmonia tristrigana Dyar, 1903, List North Amer. Lep., no. 5275.
Laspeyresia tristrigana Barnes and McDunnough, 1917, Check List Lep. Bor. Amer., no. 7220.

On the prairie lands and along the fences adjoining fields that had been planted in cotton the previous year (1917) we frequently found a white

and pinkish larva feeding on the seeds of *Baptisia* spp. about the size and with much the general appearance of the pink bollworm. Except for the complete circle of crochets on the prolegs the superficial resemblance was rather striking. The structural characters are so obviously different as to prevent confusion by a careful observer. The arrangement of setæ on the ninth abdominal segment is typically olethreutid (Compare Pl. 103, E; 105, B), and the grouping of the head setæ is also quite different from that of the pink bollworm; A^1, A^2, A^3, and L^1 lie in almost a straight line, and the puncture A^a is well back of (almost directly posterior to) A^2 rather than between it and A^1 as in *Pectinophora gossypiella*.

The larva is most like that of *Eucosma helianthana*, from which it differs in the size of the head, the color of the thoracic shield, and the position of epicranial puncture A^a. In *E. helianthana* the puncture (A^a) lies to the side directly dorsad of seta A^2, between it and the adfrontal suture, the head is smaller in the full-grown larva, and the thoracic shield is brown with a more or less distinct hyaline kidney-shaped spot on the side. In *Laspeyresia tristrigana* the shield is of the general body color with a few small, irregular, scattered yellow spots. Neither species has an anal fork.

The pupa is similar to that of *Crocidosema plebeiana*.

Several adults were reared during May, 1919, from larvæ collected in August, 1918 (Anahuac, Tex.) and in November, 1918 (El Vista, Tex.).

FAMILY PHALONIIDAE

PHALONIA CEPHALANTHANA, N. SP.

(PL. 100, A)

Phalonia cephalanthana, n. sp.

Antennæ grayish black, palpi dull yellow, whitish above and on inner sides. Face whitish. Head yellow. Thorax mahogany-red. Forewings brownish overlaid with mahogany-red mixed with a few blackish scales, the red scaling unevenly distributed, forming an obscure but distinguishable outwardly angulate basal patch, a broad, vertical, somewhat irregular median fascia, and a moderately broad, outwardly oblique costal dash near apex, the latter extending from apical fifth of costa to below middle of termen; other areas of wing brown, more or less streaked with reddish or black scales; cilia mixed brown, red, and black. Hind-wings smoky gray; underside faintly mottled; cilia grayish white. Legs heavily dusted on outer sides with grayish black; ends of joints and inner sides yellowish white. In general appearance to the naked eye the insect is a rather pale wine-red, blotched with darker shading of the same color. Male genitalia of type figured (Pl. 100, A). Alar expanse 8 to 10 mm.

HABITAT.—Shores of Lake Charlotte, Chambers County, Tex. (Heinrich).

FOOD PLANT.—*Cephalanthus occidentalis*.

TYPE.—Cat. No. 23744, United States National Museum.

Described from male type and 16 male and female paratypes reared September 16 to 24, 1919, from larvæ collected September 10, 1918; a distinct and easily recognized species.

The larva feeds in the seed pods. It is a dirty white with the chitinized areas about the body tubercles conspicuous, moderately large, round or oval, and a dull smoky fuscous, the chitinizations becoming heavier and more extended toward the caudal end; on the eighth abdominal segment paired setæ I are on a single chitinization; also paired II; on the ninth abdominal segment paired II, I, and III are on a single shield; the setal arrangement of the ninth abdominal segment is similar to that of the Olethreutidae with I and III rather closely approximate; seta III on eighth abdominal segment directly anterior to the spiracle; anal shield brown; anal fork developed, 6-pronged; crochets of prolegs uniordinal and arranged in a complete circle, 36 to 40; skin finely granulate; thoracic legs pale; thoracic shield the color of body except for a shading of yellow along hind margins. Head yellow, shading to yellowish brown; ocellar pigment slight, continuous but not filling the ocellar area; setæ of anterior and lateral group (A^1, A^2, A^3, and L^1) crowded well forward on head; A^1, A^2, and A^3 forming a slightly acute angle; L^1 closely approximate to A^3. Full-grown larva 8 to 9 mm. long.

The pupa is similar to that of *Crocidosema plebeiana* except that the caudal end is more rounded. There is no cremaster.

FAMILY AEGERIIDAE

ZENODOXUS PALMII (NEUMOEGEN)

Larunda palmii Neumoegen, 1891, *in* Ent. News, v. 2, p. 108.
Paranthrene palmii Beutenmüller, 1901, *in* Mem. Amer. Mus. Nat. Hist., v. 1, pt. 6, p. 316.
Paranthrene palmii Dyar, 1903, List North Amer. Lep., no. 4260.
Zenodoxus palmii Barnes and McDunnough, 1917, Check List Lep. Bor. Amer., no. 6735.

Several specimens of this species were reared during April and May, 1919, from larvæ collected at Brownsville, Tex., January 23 and February 3, 1919, by H. C. Hanson and E. L. Diven. The caterpillars bore in the stems of *Wissadula lozani* and are usually found well down in the stems at the base of the plants near the roots. The adults agreed very well with the description of *Zenodoxus palmii* Neum. I have since compared them with the type in the Brooklyn Institute and have little hesitation in determining them as that species, although they are a trifle small (alar expanse 17.5 to 21 mm.).

The larvæ of this family are not likely to be confused with those of the pink bollworm and are easily identified by the peculiar arrangement of the ocelli—that is, with ocelli I to IV grouped together forming a trapezoid and V and VI well separated from the other four—and the crochets of the prolegs. The latter are always uniordinal and *in two transverse* bands. The setæ on the ninth abdominal segment are much the same as in the Olethreutidae.

The pupæ have two rows of strong spines on the dorsum of several of the abdominal segments as in the Tortricidae, but the wings are narrow and pointed, the maxillary palpi are large and conspicuous, and the thoracic spiracle is normally well developed; thus they are distinguished readily enough from pupæ of the latter group.

FAMILY PTEROPHORIDAE

EDEMATOPHORUS VENAPUNCTUS, N. SP., BARNES AND LINDSEY [1]

During April and May, 1919, Mr. E. L. Diven reared eight specimens of a pterophorid moth from larvæ feeding on the leaves of a composite at Brownsville, Tex. These were referred to Mr. Lindsey, who determined them as *Oedematophorus venapunctus*, an unpublished species, which he and Dr. Barnes had recently described from collected material.

The species is not a malvaceous feeder and has no special interest here apart from the rearing record and the structural peculiarities of the larva and pupa which, while strikingly modified in this particular form, will serve, nevertheless, to exemplify the family.

The pterophorid larvæ have only two setæ on the prespiracular shield of the prothorax and setæ IV and V approximate on the proleg-bearing abdominal segments, as in the Pyralidae with which they are affiliated. They have, however, in distinction from the Pyralidae proper, long stem-like prolegs and a greater or less development of secondary setæ. The crochets are also peculiar, being uniordinal, few in number (6 to 8 in the genus Oedemataphorus), and arranged in a quarter circle opening outwardly. In *O. venapunctus* the secondary hairs are confined to a row

[1] Inasmuch as the foregoing name was desired for this paper in advance of their proposed revision of the Pterophoridae Drs. Wm. Barnes and A. W. Lindsey have kindly furnished the following description:

Oedematophorus venapunctus, n. sp., Barnes and Lindsey.

Head whitish ochreous between the antennæ, elsewhere light brown. Antennæ and palpi pale brownish ochreous, almost white, the latter short, oblique or porrect. Thorax and legs of the same shade of pale brownish ochreous, the fore and middle legs tinged with brown inside. Abdomen similar both above and below, with a fine, brown, middorsal line.

Primaries concolorous with thorax, darker toward costa, especially in first lobe, though this shade is scarcely evident in some specimens. Just before and below the base of the cleft is a small blackish brown spot, isolated except in our darkest specimen, in which it is continued obliquely toward the costa by a faint dark shade. In the outer margin of the second lobe there are four short, dark dashes on the tips of the anal, cubital, and third median veins. These are very faint in some specimens. A similar but heavier spot occurs on the inner margin of the first lobe a short distance before its apex at the tip of the fifth radial. Two vague dots sometimes appear on the costal margin of this lobe, one just before the apex and the other almost opposite the one on the inner margin. Fringes concolorous, slightly darker toward the apex of the wing and with their bases slightly paler. Secondaries somewhat paler than primaries and with a more grayish tinge. Fringes concolorous with slightly paler bases.

Expanse 15 to 18 mm.

Described from the following series: Holotype male, Brownsville, Tex., March; paratype male, same locality; allotype and six paratypes females, San Benito, Tex., March and April. (Collection Barnes).

Paratype male, Brownsville, Tex., March, and paratype female, from San Benito, Tex., April, in United States National Museum, type Cat. no. 23495.

This species appears to be allied to *Oedematophorus paleaceus*, *O. stramineus*, *O. kellicotti*, and related species. It differs from the first two in the presence of the terminal dots and from the last two in that the dot in the disc of the primaries is not contiguous to the base of the cleft. The form of the male genitalia also differs from that of any related species known to us. We have been unable to place it as a described Mexican or Central American species.

of 5 to 8 in the area normally occupied by seta VI. The body tubercles are somewhat produced, especially on the prothorax and tenth abdominal segment, and the hairs themselves are swollen and bulbous. In addition to the setæ there are on all except the first thoracic and the last abdominal segments several fingerlike projections from the skin. On the abdomen these arise back of setæ I, II, III, IV, and V from the base of their tubercles and in the area back of the spiracle and seta group IV–V. The prothorax is somewhat produced dorsally, and the head is capable of retraction under the cover of this rooflike projection.

In the pupa the venter of the eighth, ninth, and tenth segments is deeply concave with the lateral edges fringed by rather short flexible setæ. The ventral edge of the tenth segment and the anterior margins of the concavity are also armed with clusters of slender, hooked hairs. The caudal end is sharply pointed, but there is no distinct cremaster.

The larva is an external feeder, and the pupal period is very short. Larvæ collected by Diven from April 7 to 14, 1919, produced moths as early as the ninteenth of the same month.

FAMILY PYRALIDAE

SUBFAMILY THYRIDINAE

MESKEA DYSPTERARIA GROTE

(PL. 101, E, F; 104, B; 109, A–E)

Meskea dyspteraria Grote, 1877, *in* Canad. Ent., v. 9, p. 115.
Meskea dyspteraria Dyar, 1903, List North Amer. Lep., no. 4139.

This species was described by Grote from a single female collected in Bastrop County, Tex. Up to the present it has been rare in collections, Grote's type and a male from the Riley collection being the only representatives in the United States National Museum. Nothing was known of its larval habits or life history. We succeeded in rearing a large series of the moths and found their larvæ rather abundant though locally distributed. The larvæ mine the stems of several malvaceous plants, forming a conspicuous, elongate gall. The species seems to favor Malvaviscus and Abutilon; but occasional larvæ were found in galls on *Kosteletzkya* sp. (Anahuac, Tex., Aug. 13–14, 1918, More and Diven, collectors). The species overwinters as larvæ in the gallery, pupating in the spring and producing moths during April and May. From larvæ collected in *Malvaviscus drummondii* at Wallisville, Tex., September 3, 1918 (Hanson, Diven, and Heinrich), October 28, 1918 (Hunter, Busck, and Johnson), and November 5, 1918 (Barber, More, and Heinrich) moths were reared during May 9 to 25, 1919; in *M. drummondii* taken along the San Jacinto River near Crosby, Tex. (Hanson), November 6, 1918, moths issued May 4 to 10, 1918. Larvæ taken in *Abutilon berlandieri*, at Brownsville, Tex., December 31, 1918, and in *A. incanum* at Barreta, Tex., January 5,

1919 (Hanson) pupated the latter part of March and produced moths from April 5 to May 22, 1919. Neither larva nor work were found in cotton or okra or on any of the various species of Hibiscus, though there appears to be no reason why these plants should escape.

The full-grown larva is somewhat larger than a mature pink bollworm (22–22.5 mm. long) and is easily distinguished from it by the pyralid arrangement of the body setæ (*two* setæ only on prespiracular shield of prothorax and IV and V approximate on proleg-bearing abdominal segments). The structural characters of larva and pupa are fully illustrated in Plates 101, 104, and 109. These and the larval habits will serve to identify the species and distinguish it readily from any other lepidopteron of similar food plant and habits.[1]

SUBFAMILY PYRAUSTINAE

NOCTUELIA RUFOFASCIALIS (STEPHENS)

Ennychia rufofascialis Stephens, 1834, Illus. Brit. Ent., Haust, v. 4, p. 33.
Botys (?) thalialis Walker, 1859, List Lep. Brit. Mus., pt. 18, p. 582.
Noctuelia thalialis Hampson, 1899, *in* Proc. Zool. Soc. London, pt. i, p. 279, 1899.
Noctuelia thalialis Dyar, 1903, List North Amer. Lep., no. 4478.
Noctuelia rufofascialis Barnes and McDunnough, 1918, Contrib. Nat. Hist. Lep. North Amer., v. 4, no. 2, p. 167.

The larva of this species is a seed-feeder in pods of Abutilon, Wissadula, Malvastrum, Sida, and possibly other malvaceous or similar plants. It feeds in much the same way as the pink bollworm and pupates in a thin cocoon either in the empty seed pod or on the outside of the plant. Two larvæ were taken at Brownsville, Tex., April 11, 1919, by Diven feeding in the young terminal shoots of cotton. This habit, however, is unusual. When full-grown the larva is about the size of a full-fed pink bollworm and seems ridiculously large for the small seed pods within which it must accommodate itself. It is very strikingly and beautifully marked and very similar to the caterpillers of *Gelechia bosquella* Chambers and *Borkhausenia diveni*, elsewhere mentioned in this paper. It is readily distinguished from them by the pyraloid setal arrangement of the prothorax (two setæ only in the prespiracular group). The general body color is white with the thoracic segments and anterior half of the first abdominal segment a deep wine-red. The remaining abdominal segments are also partially encircled by a broad band of the same color. The head is light yellow, and the thoracic and anal shields are yellow or brownish, the legs smoky fuscous, and the crochets of the prolegs (7 to 10) *uniordinal and arranged in a circle broken outwardly* as in the pink bollworm—a very unusual structure in this subfamily.

[1] It should be noted that puncture A^a on the epicranium is somewhat differently located on different specimens, sometimes higher, sometimes lower, occasionally even lying between seta A^3 and I_1 and frequently differently placed on opposite sides of the same head. Body seta IV on abdominal segment 9 is also very often absent. When present it is always short and inconspicuous.

Adults were reared during May, 1919, from larvæ collected in pods of Abutilon and Malvastrum at Brownsville, Tex., December 27, 1918 (Hanson), and April 12, 1919 (Diven). Other larvæ were collected in seed pods of Wissadula and Sida at Brownsville, but no adults were reared. The species is not common and we found it only in the vicinity of Brownsville.

PACHYZANCLA BIPUNCTALIS (FABRICIUS)

Phalaena bipunctalis Fabricius, 1794, Ent. Syst., t. 3, pars 2, p. 232.
Pachyzancla bipunctalis Dyar, 1903, List North Amer. Lep., no. 4344.

Several moths of this species were reared September 14 to 18, 1918, from larvæ tying the terminal leaves and feeding on the seeds of the common pigweed (*Amaranthus hybridus*). Larvæ were collected at Turtle Bayou, Tex., September 4, 1918.

The caterpillars are typical Pyraustinae with the proleg crochets *triordinal* and arranged in a penellipse.

All the Pyralidae are distinguished by having *two setæ* on the prespiracular shield of the prothorax (IV and V) and IV and V approximate on the proleg-bearing abdominal segments (compare Pl. 103, B; 104, B). No other group posesses this combination.

GLYPHODES PYLOALIS WALKER

Glyphodes pyloalis Walker, 1859, List Lep. Brit. Mus., pt. 19, p. 973–974.
Glyphodes pyloalis Hampson, 1899, *in* Proc. Zool. Soc. London, 1898, pt. 4, p. 746.

On a private estate near Alto Loma, Tex., the writer found a number of pyralid larvæ tying and feeding on the leaves of a mulberry tree. A moth was reared from these which both Mr. Schaus and Dr. Dyar have determined as *Glyphodes pyloalis* Walker. This record is of interest because *G. pyloalis* Walker is a Chinese species which has not hitherto been recorded from the United States. Unfortunately as the single reared specimen is a female the genitalia could not be compared with those of oriental specimens.

The larvæ were collected September 27, 1918. All died during the winter except one which pupated about the middle of April, 1919. The moth issued April 19, 1919.

SUBFAMILY CRAMBINAE

DICYMOLOMIA JULIANALIS (WALKER)

(PL. 101, C, D; 103, B; 106, D; 108, E–H)

Cataclysta (?)julianalis Walker, 1859, List Lep. Brit. Mus., pt. 17, p. 438.
Dicymolomia julianalis Dyar, 1903, List N. Am. Lep., no. 4634.

The larva of this species is the caterpillar popularly known in the cotton areas of Texas as the "white worm" and is the one most easily and frequently confused with the pink bollworm. The two when full-grown are about the same size, and both have the crochets on the

prolegs arranged in a circle broken outwardly. *Dicymolomia julianalis* is also frequently found in cotton bolls. Its normal and favored food plant is cattail (*Typha* sp.) in the spike of which it feeds and undergoes its transformation. In some parts of Texas, however, we also found it commonly in old and diseased cotton bolls, feeding upon the lint and in some cases the cotton seeds. We did not, however, find it in any green or healthy bolls. Larvæ were collected in the region about Beaumont during November, 1918, and near Brownsville from December, 1918, until early April, 1919. Adults issued from the latter part of March until the middle of May. The species overwinters in the larval stage, the caterpillars remaining in the fallen and rotting bolls and pupating during February and early March.

While very similar in superficial appearance to the pink bollworm and easily mistaken for it by one not familiar with larval characters, the caterpillar of *Dicymolomia julianalis* is easily distinguished on structure. The position of the anterior puncture (A^a) of epicranium back of seta A^2 and the presence of *only two* setæ on the small shield anterior to the prothoracic spiracle at once separates it from Pectinophora.

The pupa is smooth except for the normal body seta and a half dozen slender hooked spines on the cremaster and is not likely to be mistaken for that of *Pectinophora gossypiella*.

The structural characters of both larva and pupa are fully figured in Plates 101, 103, 106, and 108.

SUBFAMILY PHYCITINAE

MOODNA OSTRINELLA (CLEMENS)

(PL. 104, E)

Ephestia ostrinella Clemens, 1861, *in* Proc. Acad. Sci. Phila., 1860, p. 206.
Manhatta ostrinella Hulst, 1903, *in* Dyar, List North Amer. Lep., no. 4886.
Moodna ostrinella Barnes and McDunnough, 1917, Check List Lep. Bor. Amer., no. 5795.

The larva of this species is a scavenger feeding in diseased cotton bolls in company with and in much the same manner as *Dicymolomia julianalis*. It is a smaller caterpillar (8 to 9.5 mm. long) when full-grown. The heavy, ringlike chitinization about tubercles II^b of the mesothorax and III of the eighth abdominal segment (Pl. 104, E), which is so conspicuous a feature on this and the following larva (*Homoeosoma electellum*), is a character found upon most phycitine larvæ but nowhere else, so far as I know, outside of this subfamily.

The caterpillar of *Moodna ostrinella* is a nearly uniform dirty white; thoracic shield smoky fuscous divided on dorsum by a wide median whitish line; body tubercles dark brown; skin finely granulate; body hairs moderately long, pale yellowish; legs whitish, ringed with smoky fuscous; head pale yellowish brown; labrum and anterior margins of epicranium blackish brown; ocellar pigment a black spot under each

ocellus, not continuous; crochets evenly biordinal, alternating one long and one very short hook, 40 to 44.

Larvæ collected November 24, 1918, at Kountz, Tex. Moth issued April 7, 1919.

HOMOEOSOMA ELECTELLUM (HULST)

(PL. 100, B)

Anerastia electella Hulst, 1887, *in* Entomologica Americana, v. 3, p. 137–138.
Homoeosoma electellum Hulst, 1903, *in* Dyar, List North Amer. Lep., no. 4865.

A large series of moths was reared April 23 to May 5, 1919, from larvæ collected at Brownsville, Tex., April 7, 1919, by E. L. Diven. The larvæ feed in the flower heads of a composite, making an untidy patch and eating the bloom, stem, and seeds. The species appeared to be very common.

The larva is pale smoky brown, longitudinally marked by two narrow white dorsal stripes and a similar lateral stripe; spiracles black, thoracic legs smoky fuscous; anal shield yellow, thoracic shield yellow, broadly margined laterally and posteriorly with black; head pale yellow, mottled with yellowish brown and with a broad lateral black band and a blackish shading toward anterior margins of epicranium; ocelli distinct; ocellar pigment absent or confused in the lateral black of epicranium; general structural characters as in *Moodna ostrinella;* width 6 to 7 mm.

The interesting and rather complicated genitalia of the male adult are figured in Plate 100, B.

SUBFAMILY CHRYSAUGINAE

CLYDONOPTERON TECOMAE RILEY

Clydonopteron tecomae Riley, 1880, *in* Amer. Ent., v. 3, no. 12, p. 288.
Salobrana tecomae Dyar, 1903, List North Amer. Lep., no. 4526.
Clydonopteron tecomae Barnes and McDunnough, 1917, Check List Lep. Bor. Amer., no. 5283.

The larva of this species feeds only in the seed pods of the trumpet-flower vine (*Tecoma radicans*). It is mentioned here only because its host plant is often found in the neighborhood of the cotton fields and for that reason it might be confused by the uncritical with the larva of *Pectinophora gossypiella.* It is easily distinguished, however. The spiracles are rather large, oval, and black, the edges are heavily chitinized, and the spiracle on the eighth abdominal segment is somewhat larger but no higher on the body than the others; the proleg crochets are arranged as in the Aegeriidae—that is, uniordinal and in two transverse bands—and the prothorax has only *two* setæ on the chitinization before the spiracle as in other Pyralidae. It pupates in a cocoon within the seed pod.

Moths were reared by us August 30 to September 15, 1918, from larvæ collected earlier in August (Anahuac, Tex.) the same year.

FAMILY NOCTUIDAE

Several species of this family feed upon cotton and malvaceous plants. They are easily distinguished from the pink bollworm or larvæ of any of the other groups treated in this paper by the arrangement of the body setæ and the crochets of the prolegs. Like the Pyralidae they have only two setae (IV and V) on the prespiracular shield of the prothorax, but the position of IV and V on the proleg-bearing segments is quite different, IV being *remote* from V and directly back of the spiracle (Pl. 103, D). The crochets of the prolegs are also arranged in a mesoseries (Pl. 106, E).

The following species were reared.

SUBFAMILY AGROTINAE

HELIOTHIS (CHLORIDEA) OBSOLETA (FABRICIUS)

(PL. 103, D; 106, E)

Bombyx obsoleta Fabricius, 1793, Ent. Syst., t. 3, pars. 1, p. 456.
Heliothis armiger Dyar, 1903, List North Amer. Lep., no. 2300.
Chloridea obsoleta Hampson, 1903, *in* Cat. Lep. Phal. Brit. Mus., v. 4, p. 45, 657.
Heliothis obsoleta Barnes and McDunnough, 1917, Check List Lep. Bor. Amer., no. 1090.

This species is commonly known as the "corn earworm" or "cotton bollworm." It feeds on a number of plants and often attacks cotton, doing serious damage in some localities. The larva bores into the bolls, making a large hole and destroying lint and seeds.

One moth was reared from a larva feeding on the leaves of *Malvaviscus drummondii* at Brownsville, Tex. A larva was collected by E. L. Diven, May 7, 1919. The adult emerged May 29 of the same year.

HELIOTHIS (CHLORIDEA) VIRESCENS (FABRICIUS)

Noctua virescens Fabricius, 1781, Spec. Insect., t. 2, p. 216.
Chloridea virescens Dyar, 1903, List North Amer. Lep., no. 2296.
Chloridea virescens Hampson, 1903, *in* Cat. Lep. Phal. Brit. Mus., v. 4, p. 48.
Heliothis virescens Barnes and McDunnough, 1917, Check List Lep. Bor. Amer., no. 1091.

This species has very much the same habits as *Heliothis obsoleta* Fabricius. Moths were reared September 8 and 17, 1919, from larvæ taken feeding on seeds in okra pods August 19, 1918, at Double Bayou, Tex. (E. L. Diven).

SUBFAMILY ACRONYCTINAE

BAGISARA RECTIFASCIA (GROTE)

Schinia rectifascia Grote, 1874, *in* Proc. Boston Soc. Nat. Hist., v. 16, 1873/74, p. 242.
Athethmia rectifascia Dyar, 1903, List North Amer. Lep., no. 2267.
Bagisara rectifascia Hampson, 1910, *in* Cat. Lep. Phal. Brit. Mus., v. 9, p. 156.

One moth was reared September 1 and one September 23, 1918, from larvæ collected on *Malvaviscus drummondii* August 10, 1918 (Anahuac, Tex., J. D. More). Dr. Dyar, who determined the Noctuidae, informs me that the larva of this species has not been described. Unfortunately those preserved with the foregoing experiment are Catocalinae of some kind and probably have no connection with the adults reared.

SUBFAMILY EREBINAE

ALABAMA ARGILLACEA (HÜBNER)

Aletia argillacea Hübner, 1820, Zutr. Samml. Exot. Schmett., fig. 399.
Alabama argillacea Dyar, 1903, List North Amer. Lep., no. 2555.

Several moths were reared from larvæ feeding on the cotton leaves. Larvæ were taken September 25, 1918, at Dickinson, Tex., and moths early in October of the same year. The species pupates within the folded leaves on the plant.

ANOMIS EXACTA HÜBNER

Anomis exacta Hübner, 1810, Samml. Exot. Schmett., v. 2, pl. 411.
Anomis exacta Dyar, 1903, List North Amer. Lep., no. 2557.

One moth was reared September 1, 1918, from a larva collected on *Malvaviscus drummondii*, Anahuac, Tex., August 14, 1918 (J. D. More). The larva pupated August 21, spinning a loose tie of several leaves.

ANOMIS EROSA HÜBNER

Anomis erosa Hübner, 1818, Zutr. Samml. Exot. Schmett., fig. 287.
Anomis erosa Dyar, 1903, List North Amer. Lep., no. 2556.

One moth from Brownsville, Tex., January 19, 1919, was reared from a pupa in the tied leaves of *Abutilon incanum* (H. C. Hanson, collector).

FAMILY LYCAENIDAE

STRYMON MELINUS HÜBNER

Strymon melinus Hübner, 1818, Zutr. Exot. Schmett., fig. 121.
Uranotes melinus Dyar, 1903, List North Amer. Lep., no. 335.
Strymon melinus Barnes and McDunnough, 1917, Check List Lep. Bor. Amer., no. 352.

This caterpillar feeds on a great variety of plants, including practically all the Malvaceae. On cotton it attacks the flowers and bolls, boring into the latter and feeding upon lint and seeds and making, when half-

grown, a hole which reminds one very much of the exit hole made by a pink bollworm.

The larva itself looks nothing like any of the others here treated. It is spindle-shaped, sharply tapering at each end, broad in the middle in proportion to its length, with a small head, the body covered with fine stiff secondary hairs, and greenish yellow in color.

In addition to cotton we find it frequently on okra, *Kosteletzkya* spp., *Malvaviscus drummondii*, and *Hibiscus* spp. On these it fed on the seeds, boring into the seed pods, or upon the blossoms.

The table of larval characters will serve to place the forms here treated. The characters given are not to be understood as diagnostic in all cases. In the Cosmopterygidae, for example, seta I is often as far from III as it is from II as in the Gelechiidae or the Oecophoridae. There are also a few exceptions to the gelechiid character (the remoteness of epicranial seta L^1 from A^3). The characters hold, however, for all the species here treated occurring on Malvaceae.

Characters of larvæ likely to be confused with the pink bollworm

1. Body depressed and spindle-shaped, covered with secondary setæ..LYCAENIDAE.
   Body otherwise..... 2
2. Setae IV and V on proleg-bearing abdominal segments closely approximate.. 3
   Setae IV and V on proleg-bearing abdominal segments well-separated..... 13
3. Prespiracular shield of prothorax bearing two setæ only..... 4
   Prespiracular shield of prothorax bearing three setæ..... 5
4. Prolegs long and slender; body of larvæ normally with one or more secondary setæ.....PTEROPHORIDAE.
   Prolegs otherwise; body with only primary setæ.....PYRALIDAE.
5. Body with one or more secondary setæ.....ETHMIIDAE.
   Body with only primary setæ..... 6
6. Ocelli I to IV grouped together, forming a trapezoid; ocelli V and VI fairly close together but well-separated from the other four.....AEGERIIDAE.
   Ocelli otherwise..... 7
7. Paired dorsal setæ II on ninth abdominal segment closer together than paired I on dorsum of eighth abdominal segment; usually on a single chitinization..... 8
   Paired dorsal setæ II on ninth abdominal segment at least as far apart as paired I on eighth abdominal segment and not on a single chitinization.. 9
8. Setæ I and III closely approximate on ninth abdominal segment..... 10
   Setæ I and III not closely approximate on ninth abdominal segment TORTRICIDAE.
9. Epicranial seta L^1 remote from A^3 (farther from A^3 than A^3 is from A^2) GELECHIIDAE.
   Epicranial seta L^1 approximate to A^3, at least no farther from A^3 than A^3 is from A^2..... 11
10. Seta II^a on prothorax higher than I^a.....COSMOPTERYGIDAE. (in part: *Pyroderces rileyi*).
    Seta II^a on prothorax not higher than I^a.....OLETHREUTIDAE. PHALONIIDAE.
11. Prothoracic legs very close together, coxæ touching.....BLASTOBASIDAE.
    Prothoracic legs appreciably separated..... 12

12. Setæ III on abdominal segments I to VII antero-dorsad of and close to the spiracle ..... STENOMIDAE.
Setæ III on abdominal segments I to VII dorsad of the spiracle; if occasionally somewhat antero-dorsad not close to the spiracle ..... OECOPHORIDAE.

13. Seta IV directly behind the spiracle on proleg-bearing abdominal segments; crochets of prolegs arranged in a mesoseries; two setæ only on prespiracular shield of prothorax; no secondary hair on body or head ..... NOCTUIDAE. (in part, as here represented).

PLATE 93

Male genitalia (Gelechiidae):

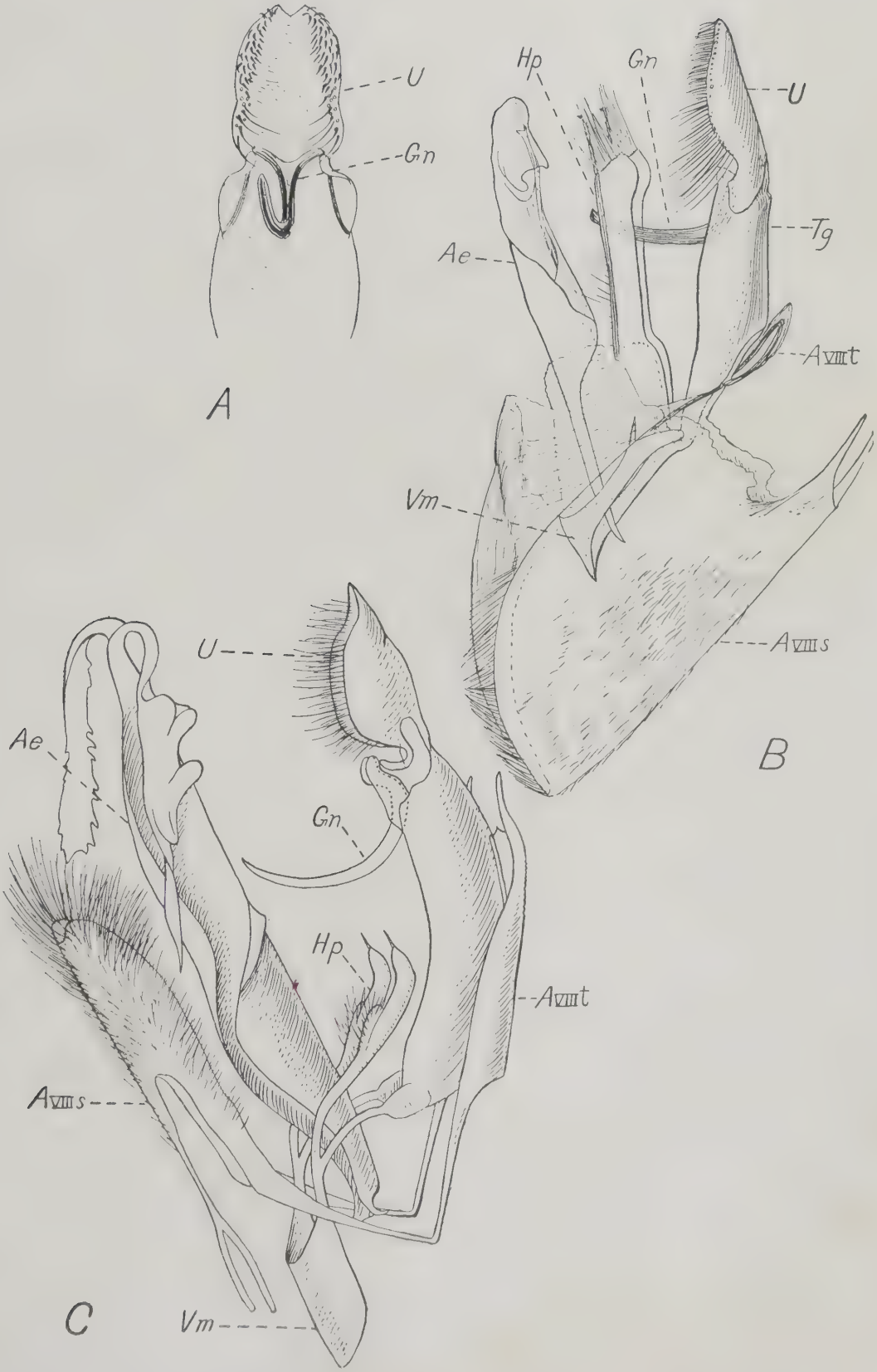
A.—*Gelechia trophella:* Posterior part of tegumen, showing uncus and gnathos, ventral view.

B.—*G. trophella:* Lateral view of male genitalia with eighth abdominal segment attached.

C.—*G. hibiscella:* Lateral view of male genitalia with eighth abdominal segment attached.

Explanation of symbols applied to male genital organs on Plates 93–100.

Ae=aedoeagus (outer chitinous sheath of penis).
An=anellus (chitinous support of aedoeagus).
Ao=opening in tegumen through which anal tube passes.
Cl=clasper on harpe.
Cn=cornutus (cornuti) spine or spines on penis proper.
Cs=cucullus of harpe.
Gn=gnathos.
Hp=harpe.
Si=soci.
Tg=tegumen.
Ts=transtilla (a costal bridge, or sometimes elements thereof not united; connecting the harpes).
Vm=vinculum.
U=uncus.
A VIIIs=sternite of eighth abdominal segment.
A VIIIt=tergite of eighth abdominal segment.


U
Gn
A
Hp
Gn
U
Tg
Ae
AVIIIt
Vm
AVIIIs
B
U
Ae
Gn
Hp
AVIIIt
AVIIIs
C
Vm

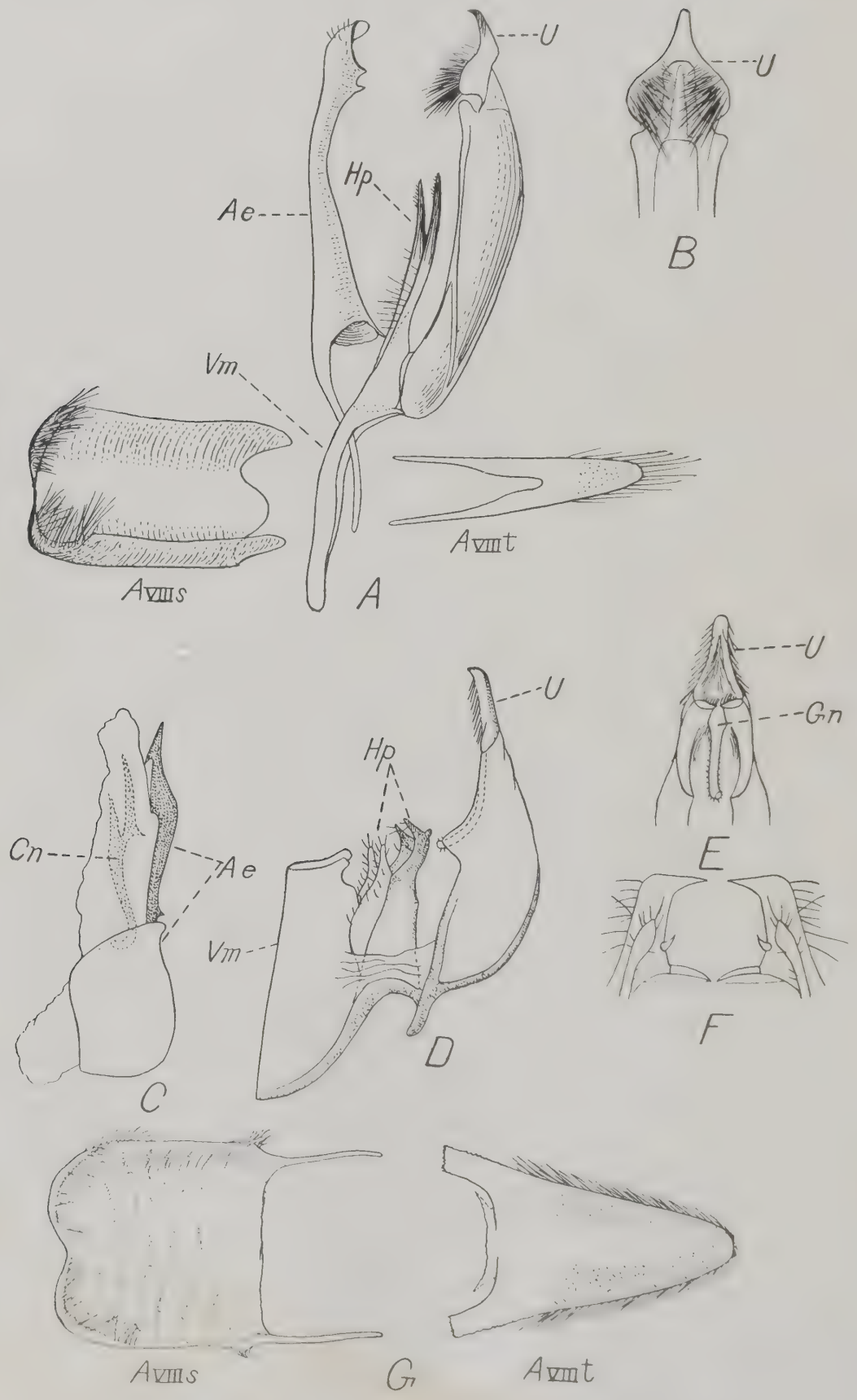

U
Hp
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PLATE 94

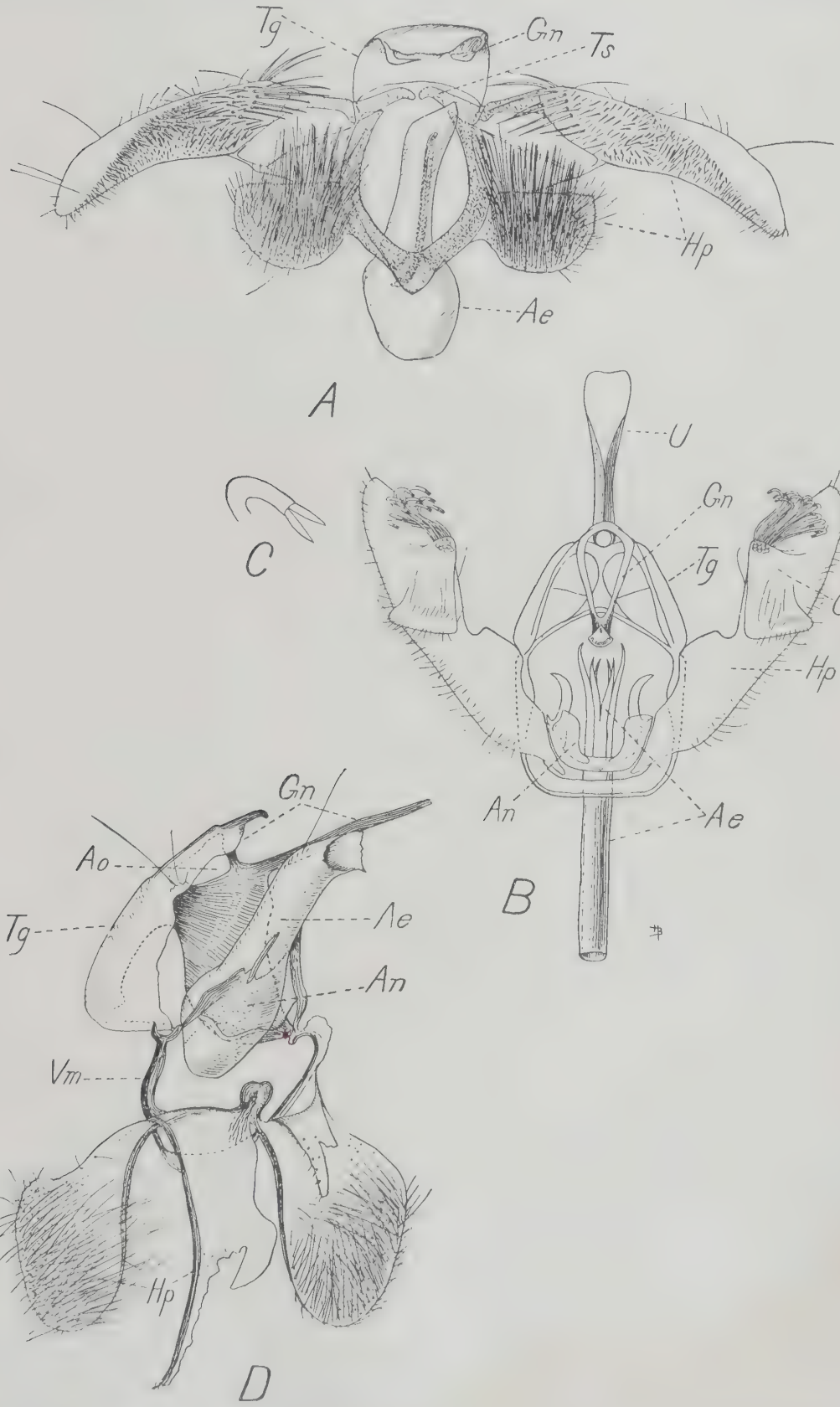
Male genitalia (Gelechiidae):

A.—*Telphusa mariona* (type): Lateral view of male genitalia.
B.—*T. mariona* (type): Posterior part of tegumen, showing uncus, ventral view.
C.—*Gelechia neotrophella* (type): Aedoeagus and penis.
D.—*G. neotrophella* (type): Lateral view of male genitalia with ædoeagus and eighth segment removed.
E.—*G. neotrophella* (type): Posterior part of tegumen, showing uncus and gnathos, ventral view.
F.—*G. neotrophella* (type): Posterior half of harpes, ventral view.
G.—*G. neotrophella* (type): Sternite and tergite of modified eighth abdominal segment.

PLATE 95

Male genitalia (Gelechiidae, Stenomidae, and Oecophoridae):

A.—*Isophrictis similiella:* Ventral view of male genitalia, spread.
B.—*Aedemoses haesitans:* Ventral view of male genitalia, spread.
C.—*A. haesitans:* Enlargement of typical split hair on cucullus.
D.—*Borkhausenia fasciata:* Ventro-lateral view of male genitalia, spread, showing asymmetrical armlike projections from gnathos and costa of harpes.


Tg
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Ts
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Ae
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Vm
Hp
D

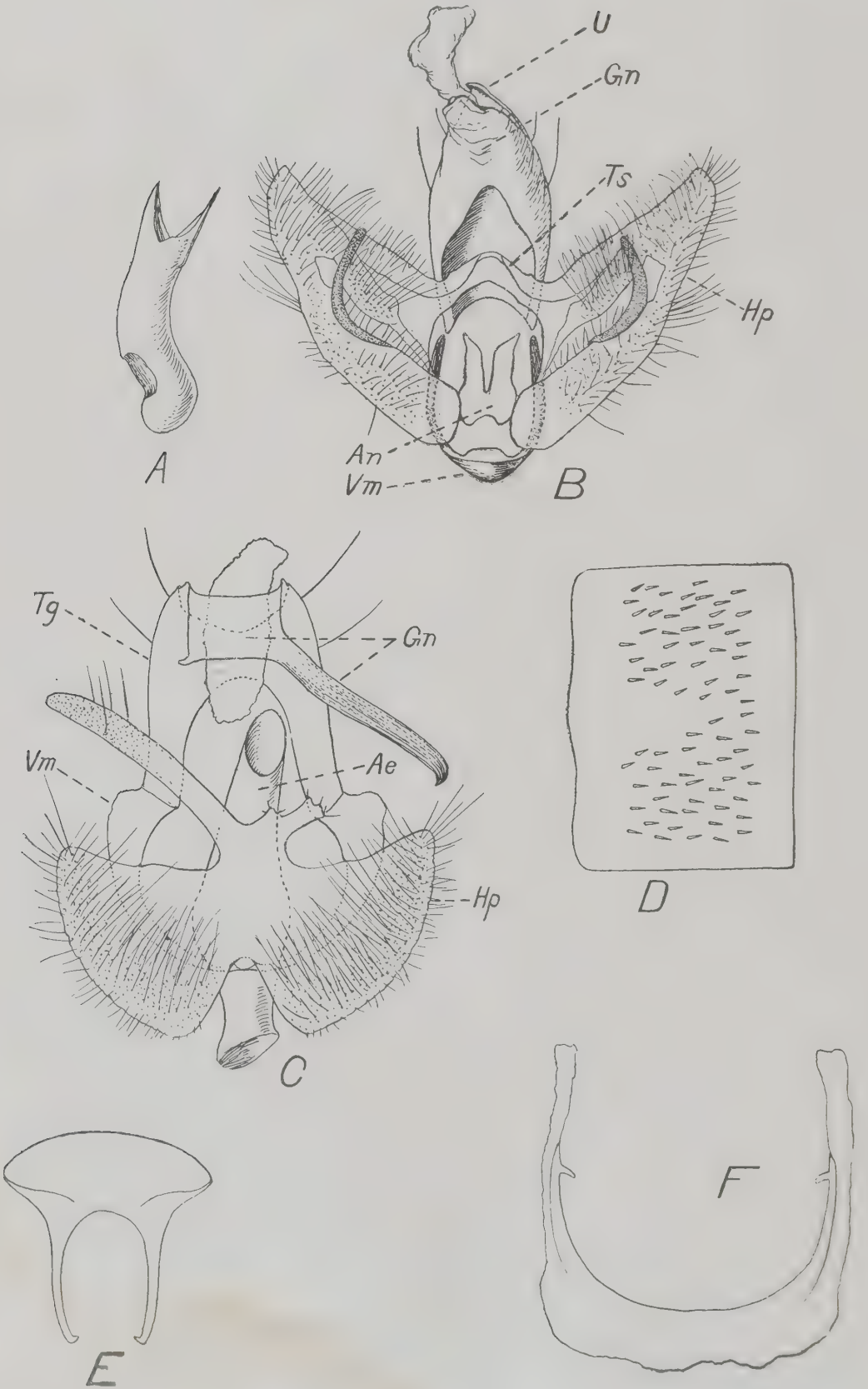

U
Gn
Ts
Hp
A
An
Vm
B
Tg
Gn
Vm
Ae
Hp
C
D
E
F

PLATE 96

Male genitalia (Oecophoridae):

A.—*Borkhausenia minutella:* Aedoeagus.
B.—*B. minutella:* Ventral view of male genitalia, spread, aedoeagus omitted.
C.—*B. diveni* (type): Ventral view of male genitalia, spread.
D.—*B. diveni* (type): Dorsal view of an abdominal segment showing spinose condition of abdomen.
E.—*B. diveni* (type): Modified tergite of eighth abdominal segment.
F.—*B. diveni* (type): Modified sternite of eighth abdominal segment.

PLATE 97

Male genitalia (Oecophoridae):

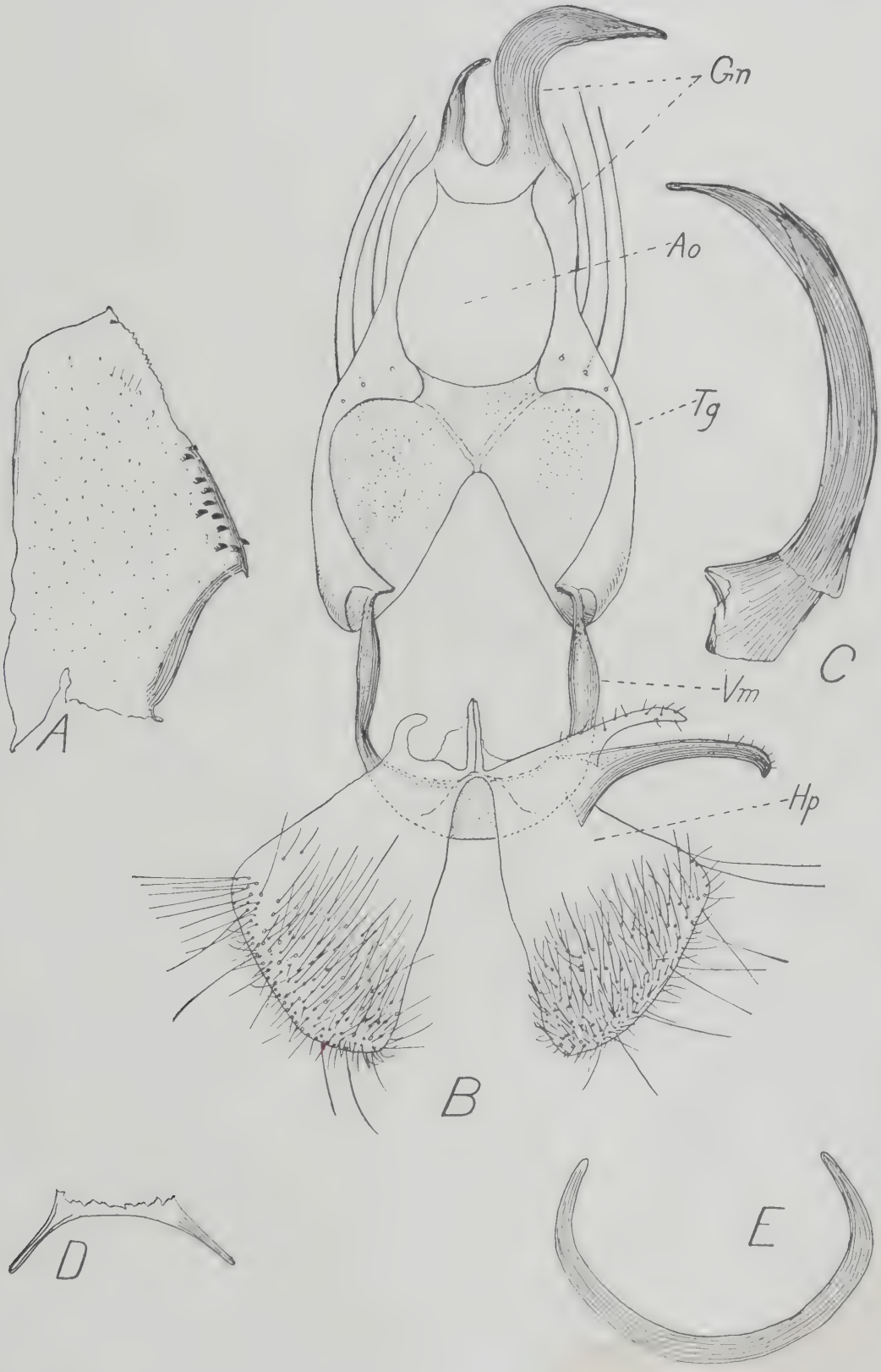
A.—*Borkhausenia conia:* Portion of tergite of seventh abdominal segment, showing spinose and chitinized character of caudal margin.

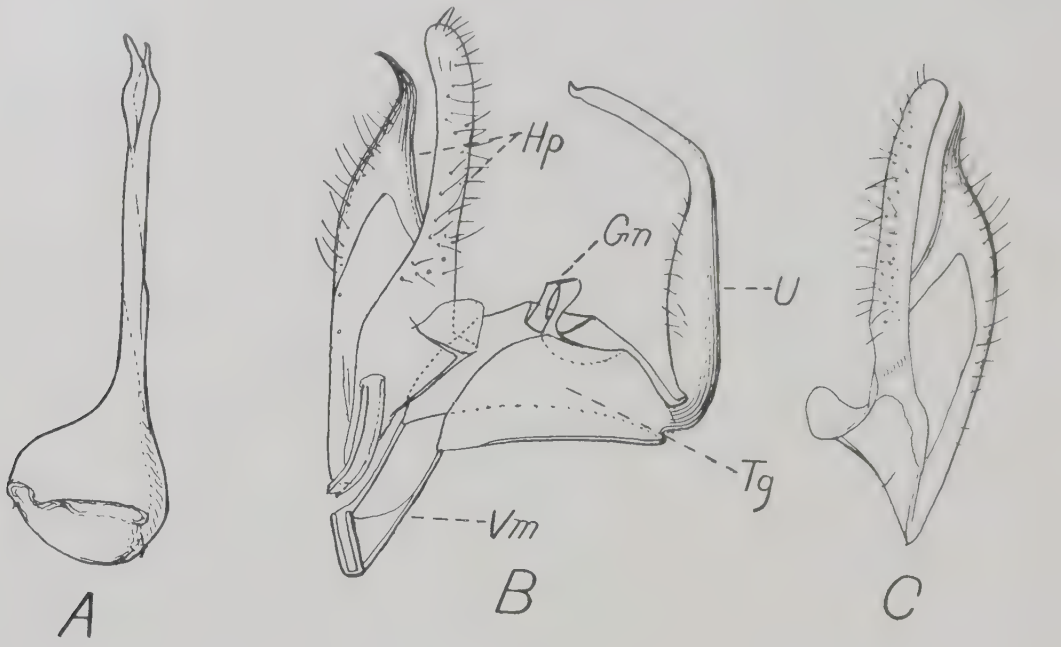
B.—*B. conia:* Ventral view of male genitalia, spread, aedoeagus omitted.

C.—*B. conia:* Aedoeagus.

D.—*B. conia:* Modified tergite of eighth abdominal segment.

E.—*B. conia:* Modified sternite of eighth abdominal segment.


Gn
Ao
Tg
Vm
Hp
A
B
C
D
E


Hp
Gn
U
Tg
Vm
A
B
C

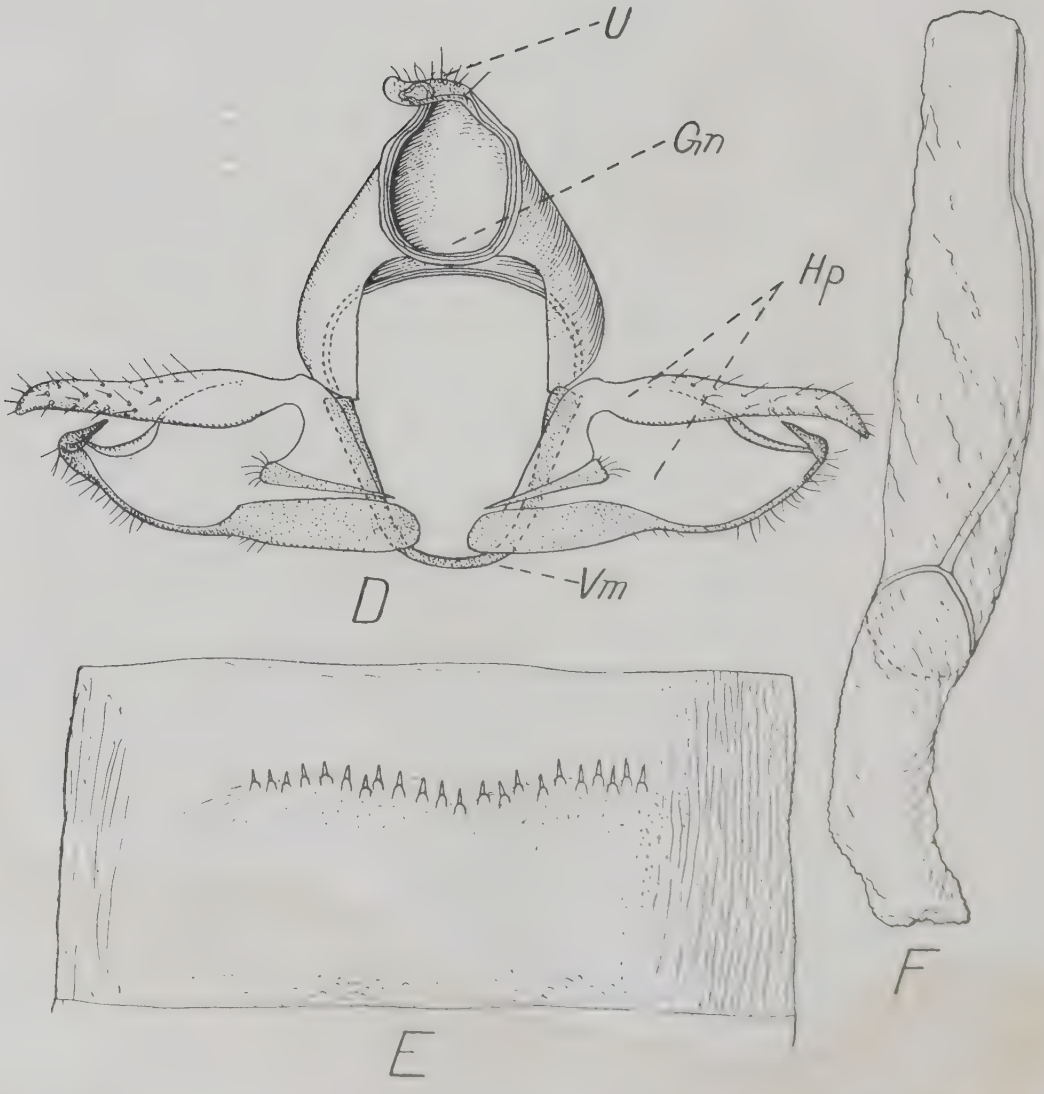

U
Gn
Hp
Vm
D
E
F

PLATE 98

Male genitalia (Blastobasidae):

A.—*Zenodochium citricolella:* Aedoeagus.
B.—*Z. citricolella:* Lateral view of male genitalia, right harpe and aedoeagus omitted.
C.—*Z. citricolella:* Right harpe.
D.—*Holcocera ochrocephala:* Ventral view of male genitalia, spread, aedoeagus omitted.
E.—*H. ochrocephala:* Dorsum of an abdominal segment showing transverse row of spines.
F.—*H. ochrocephala:* Aedoeagus and penis.

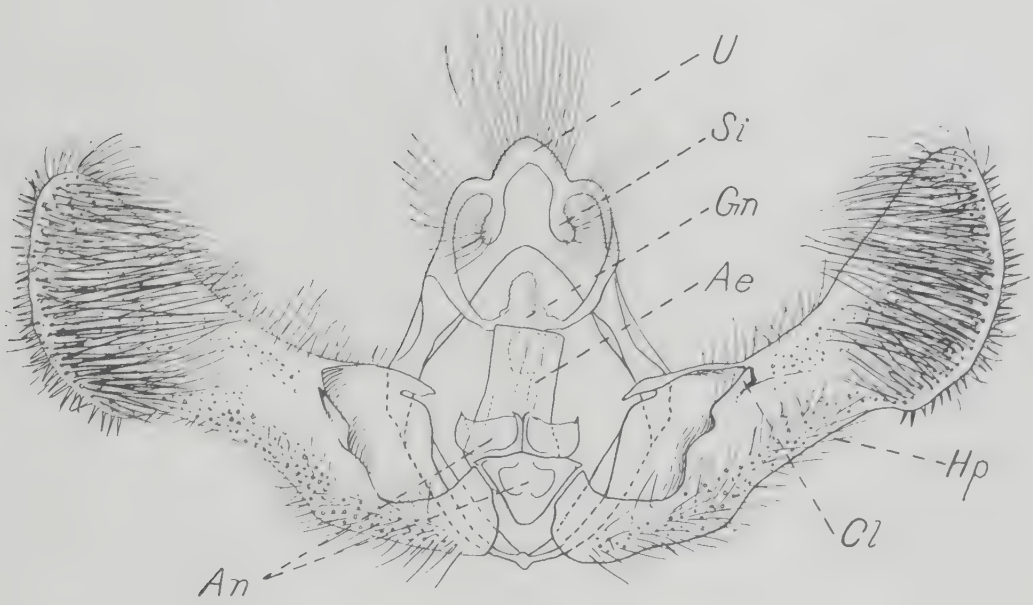
PLATE 99

Male genitalia (Olethreutidae and Blastobasidae):

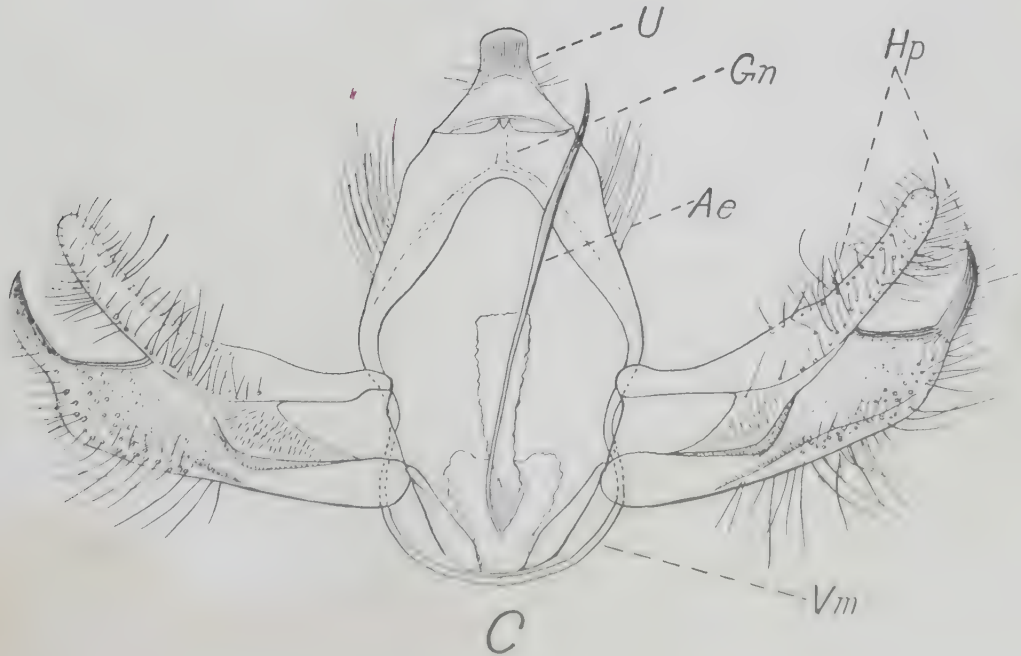
A.—*Crocidosema plebeiana:* Ventral view of male genitalia, spread.
B.—*Eucosma discretivana* (type): Ventral view of male genitalia, spread.
C.—*Holcocera confamulella* (type): Ventral view of male genitalia, spread.



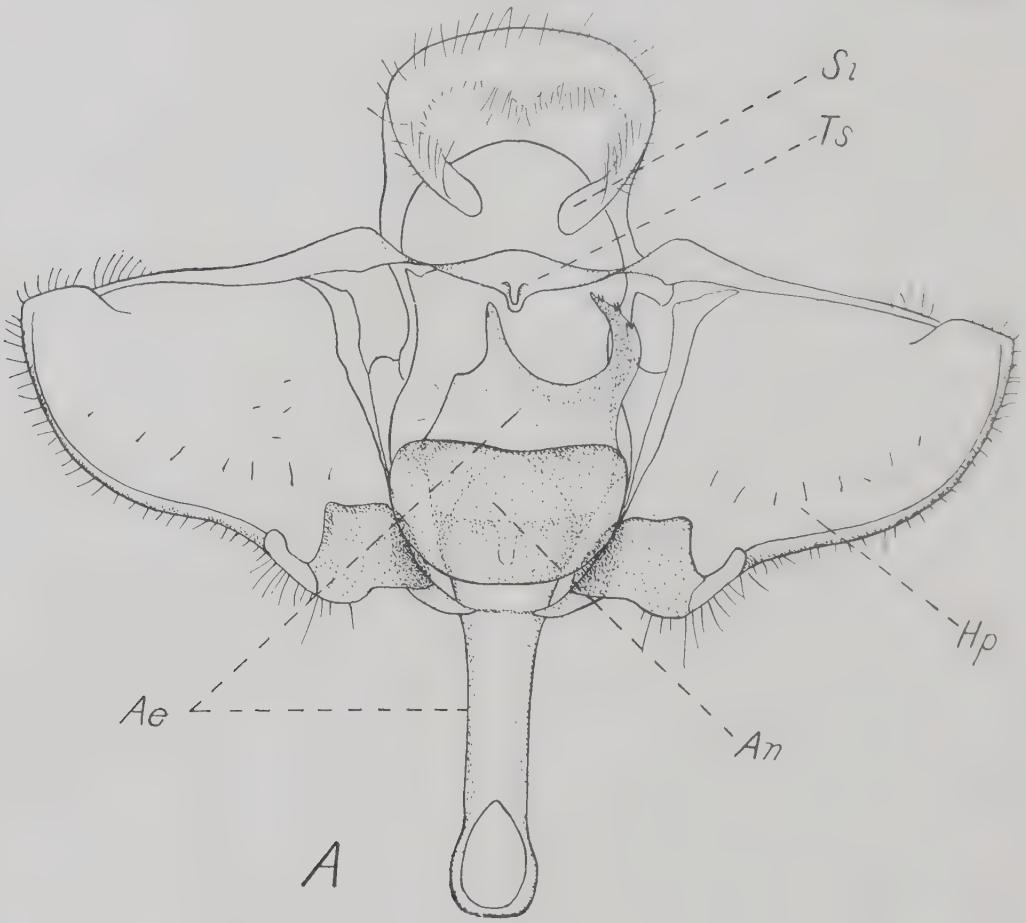
A



B



C


Si
Ts
Hp
Ae
An
A

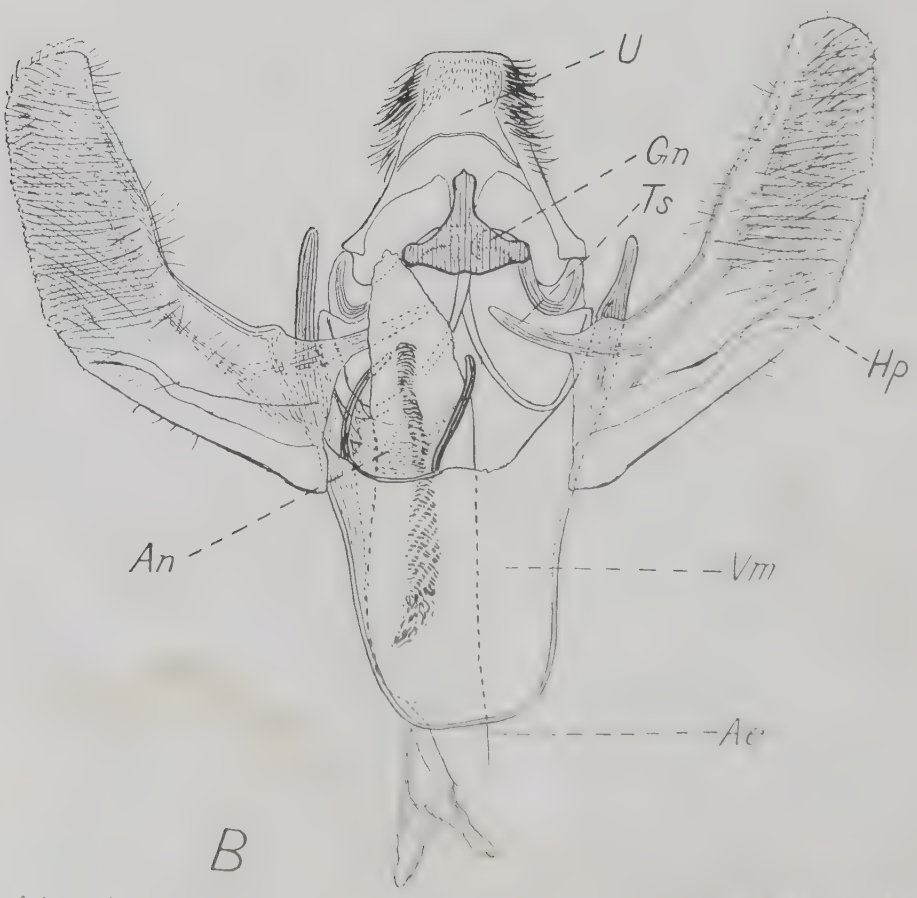

U
Gn
Ts
Hp
An
Vm
Ae
B

PLATE 100

Male genitalia (Phaloniidae and Pyralidae):

A.—*Phalonia cephalanthana* (type): Ventral view of male genitalia, spread.
B.—*Homoeosoma electellum:* Ventral view of male genitalia, spread.

PLATE 101

Larval structures:

A.—*Pectinophora gossypiella:* Head capsule, dorsal view, showing arrangement of setæ.

B.—*P. gossypiella:* Head capsule, lateral view, showing arrangement of setæ.

C.—*Dicymolomia julianalis:* Head capsule, dorsal view, showing arrangement of setæ.

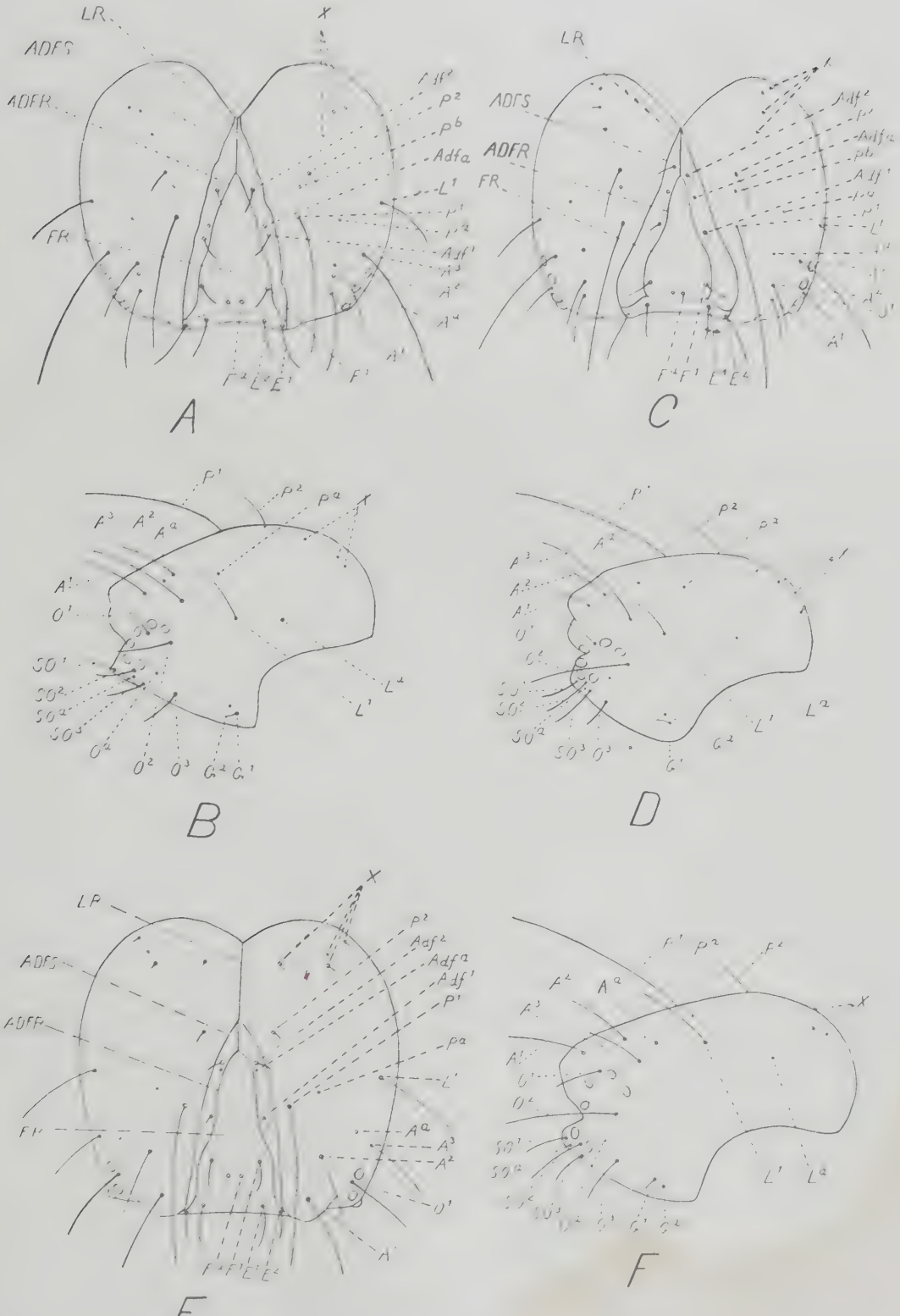
D.—*D. julianalis:* Head capsule, lateral view, showing arrangement of setæ.

E.—*Meskea dyspteraria:* Head capsule, dorsal view, showing arrangement of setæ.

F.—*M. dyspteraria:* Head capsule, lateral view, showing arrangement of setæ.

Explanation of symbols applied to larvæ on Plates 101–106.

A^1, A^2, A^3, A^a=anterior setæ and puncture of epicranium.
Adf^1, Adf^2, Adf^a=adfrontal setæ and puncture of epicranium.
ADFR=adfrontal ridge of frons.
ADFS=adfrontal suture.
AF=anal fork.
E^1, E^2=epistomal setæ.
F^1, F^a=frontal seta and puncture.
FR=frons.
G^1, G^a=genal seta and puncture of epicranium.
L^1, L^a=lateral seta and puncture of epicranium.
LR=longitudinal ridge of frons.
O^1, O^2, O^3, O^a=ocellar setæ and puncture of epicranium.
P^1, P^2, P^a, P^b=posterior setæ and punctures of epicranium.
SMp=platelike chitinization on submentum.
SO^1, SO^2, SO^3, SO^a=subocellar setæ and puncture of epicranium.
X=Ultraposterior setæ and punctures of epicranium.


A
C
B
D
E
F

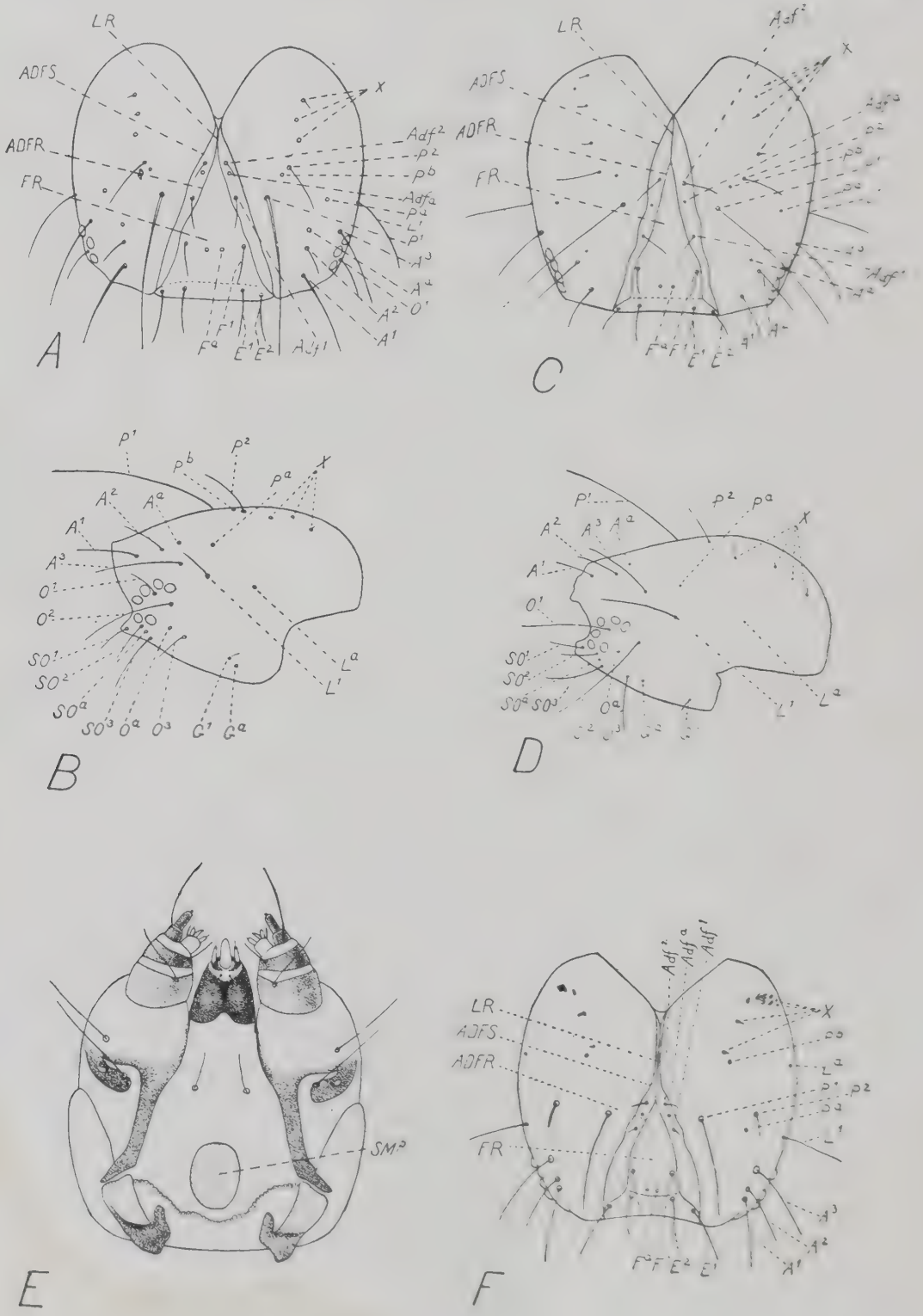

LR
ADFS
ADFR
FR
X
A
B
C
D
E
F
SM3

PLATE 102

Larval structures:

A.—*Pyroderces rileyi:* Head capsule, dorsal view, showing arrangement of setæ.
B.—*P. rileyi:* Head capsule, lateral view, showing arrangement of setæ.
C.—*Crocidosema plebeiana:* Head capsule, dorsal view, showing arrangement of setæ.
D.—*C. plebeiana:* Head capsule, lateral view, showing arrangement of setæ.
E.—*Zenodochium citricolella:* Labium and maxillæ.
F.—*Isophrictis similiella:* Head capsule, dorsal view, showing arrangement of setæ.

PLATE 103

Larval structures:

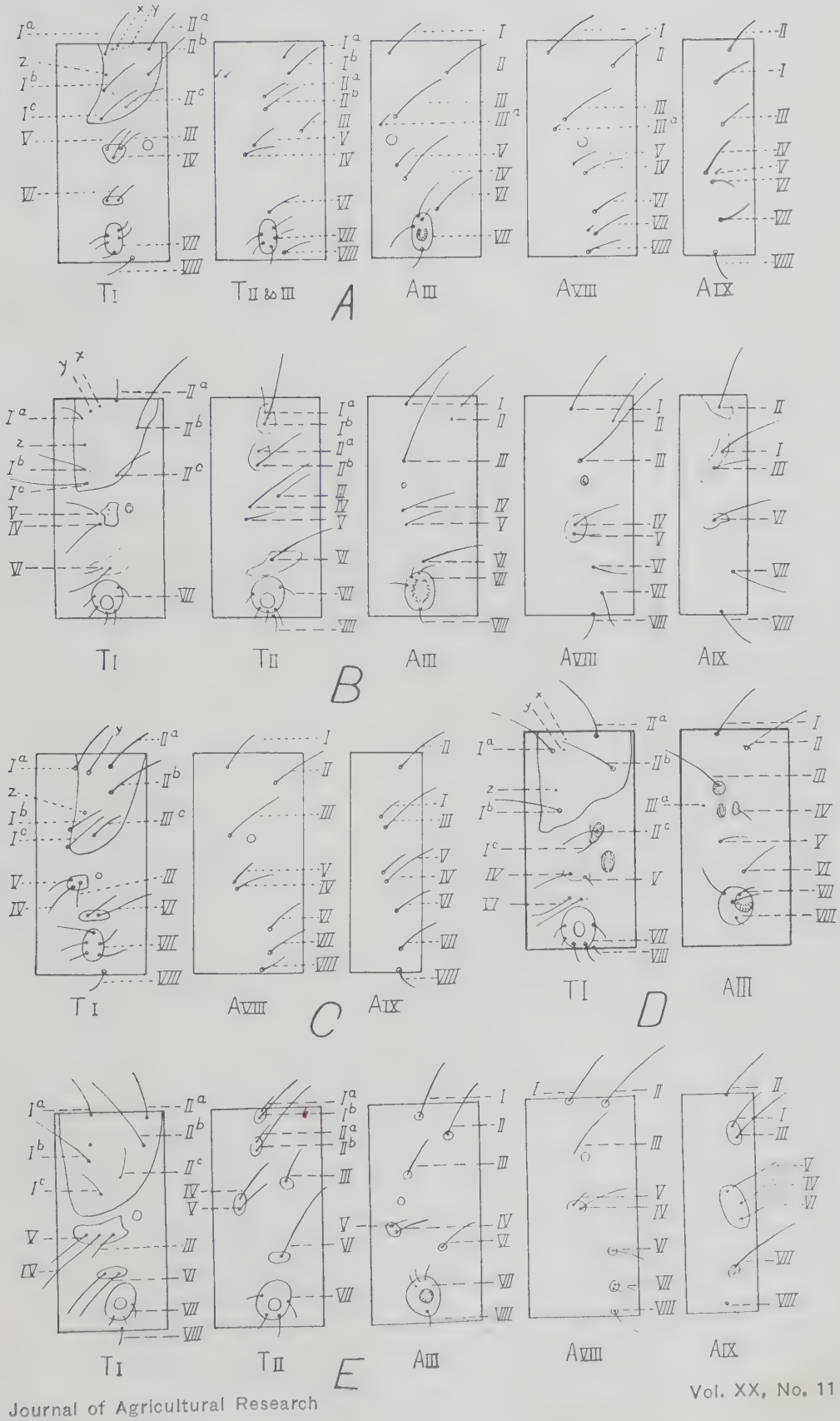
A.—*Pectinophora gossypiella:* Setal maps of first and second thoracic and third, eighth, and ninth abdominal segments.

B.—*Dicymolomia julianalis:* Setal maps of first and second thoracic and third, eighth, and ninth abdominal segments.

C.—*Pyroderces rileyi:* Setal maps of first thoracic and eighth and ninth abdominal segments.

D.—*Heliothis obsoleta:* Setal maps of first thoracic and third abdominal segments.

E.—*Crocidosema plebeiana:* Setal maps of first and second thoracic and third, eighth, and ninth abdominal segments.


A
B
C
D
E

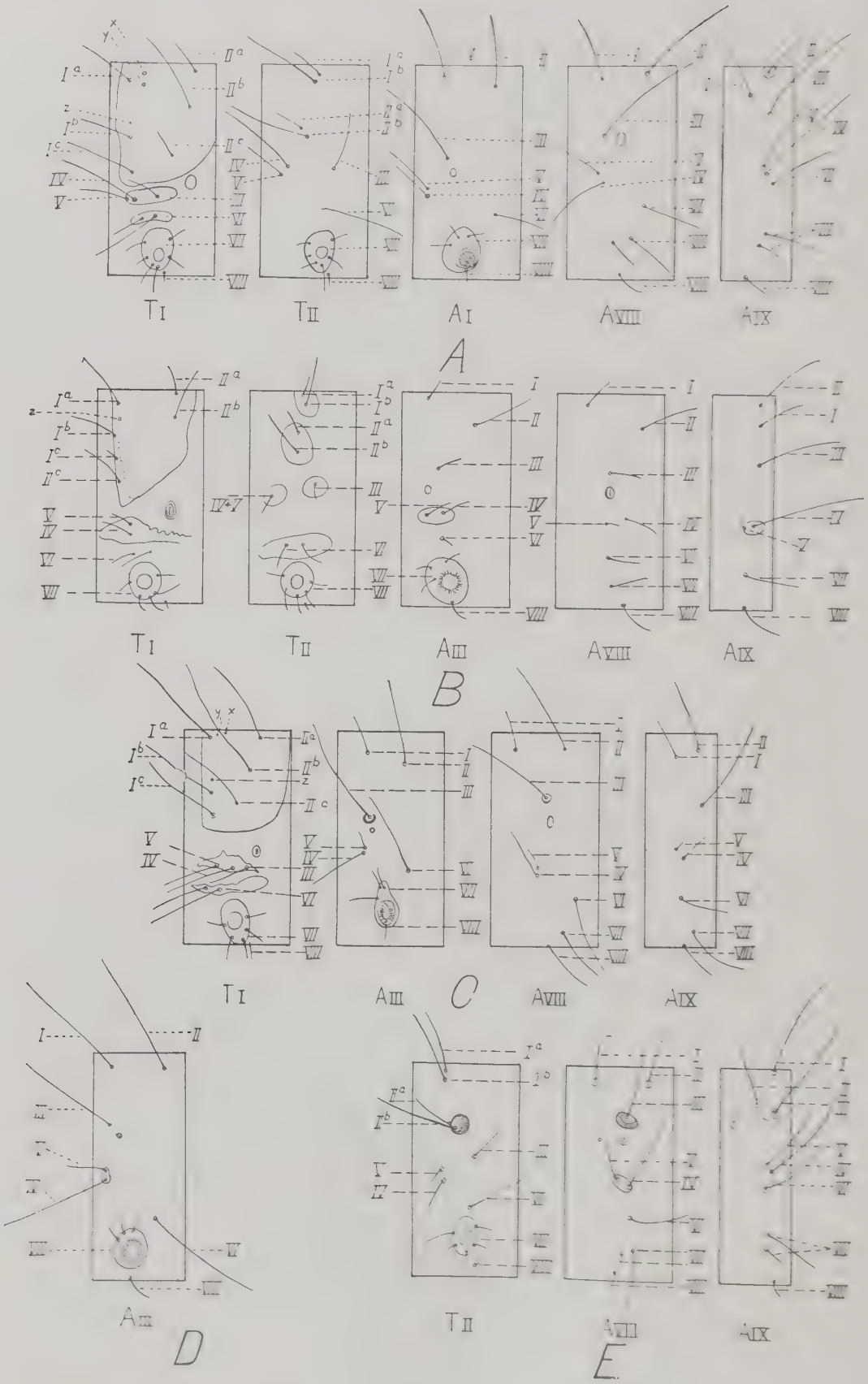

TI
TII
AI
AVIII
AIX
A
TI
TII
AIII
AVIII
AIX
B
TI
AIII
C
AVIII
AIX
AIII
D
TII
AVIII
AIX
E

PLATE 104

Larval structures:

A.—*Platynota rostrana:* Setal maps of first and second thoracic and third, eighth, and ninth abdominal segments.

B.—*Meskea dyspteraria:* Setal maps of first and second thoracic and third, eighth, and ninth abdominal segments.

C.—*Zenodochium citricolella:* Setal maps of first thoracic and third, eighth, and ninth abdominal segments.

D.—*Aedemoses haesitans:* Setal map of third abdominal segment.

E.—*Moodna ostrinella:* Setal maps of second thoracic and eighth and ninth abdominal segments.

PLATE 105

Larval structures:

A.—*Platynota rostrana:* Setal maps of eighth and ninth abdominal segments, dorsal view.

B.—*Eucosma helianthana:* Setal maps of eighth and ninth abdominal segments, dorsal view.

C.—*Pectinophora gossypiella:* Setal maps of eighth and ninth abdominal segments, dorsal view.

D.—*Pyroderces rileyi:* Setal maps of eighth and ninth abdominal segments, dorsal view.

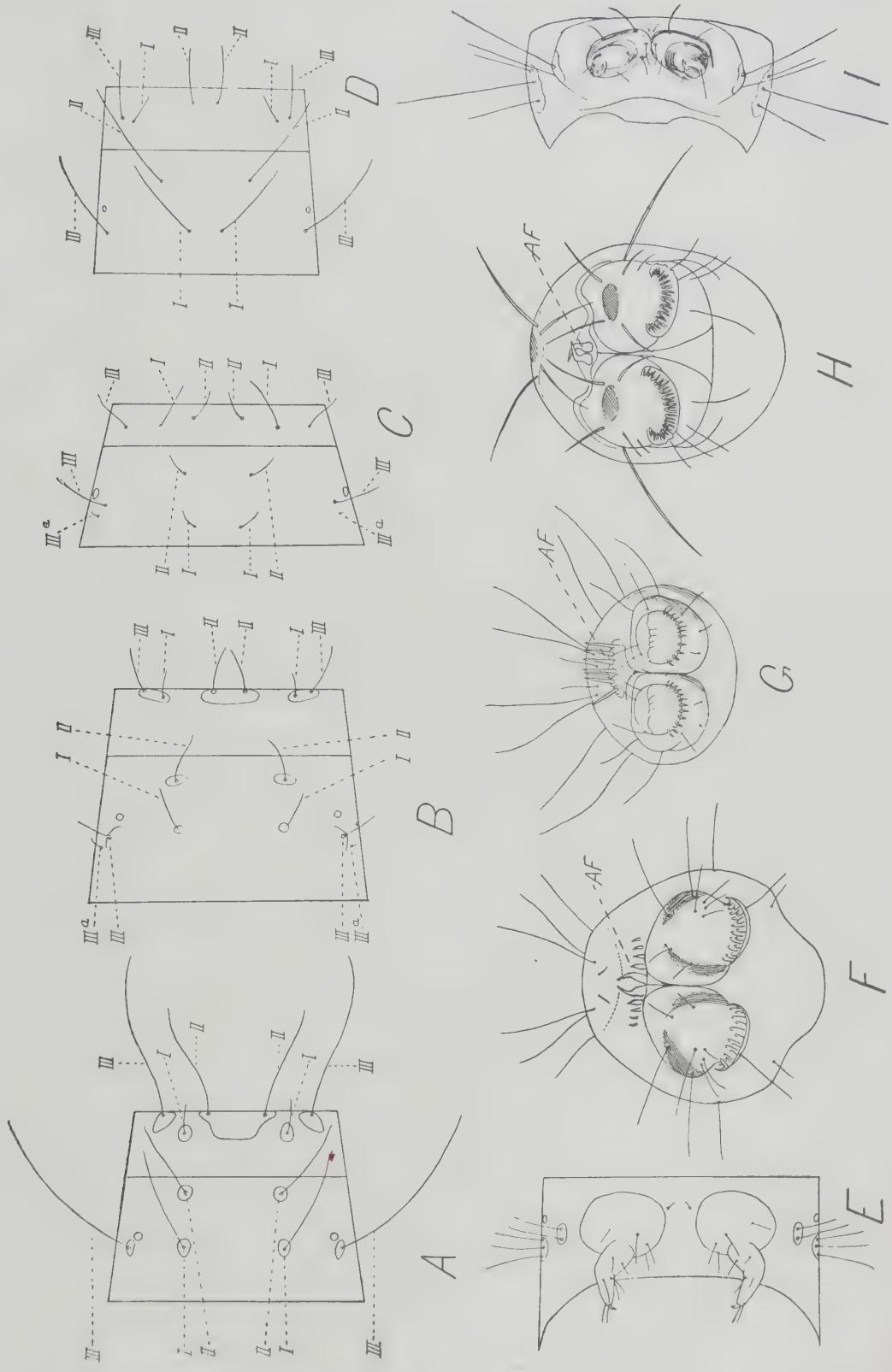
E.—*Pectinophora gossypiella:* Prothorax, ventral view, showing position of legs.

F.—*Telphusa mariona:* Ventro-caudal view of tenth abdominal segment, showing anal fork.

G.—*Crocidosema plebeiana:* Ventro-caudal view of tenth abdominal segment, showing anal fork.

H.—*Gelechia neotrophella:* Ventro-caudal view of tenth abdominal segment, showing anal fork.

I.—*Zenodochium citricolella:* Prothorax, ventral view, showing position of legs.


A
B
C
D
E
F
G
H
I
AF

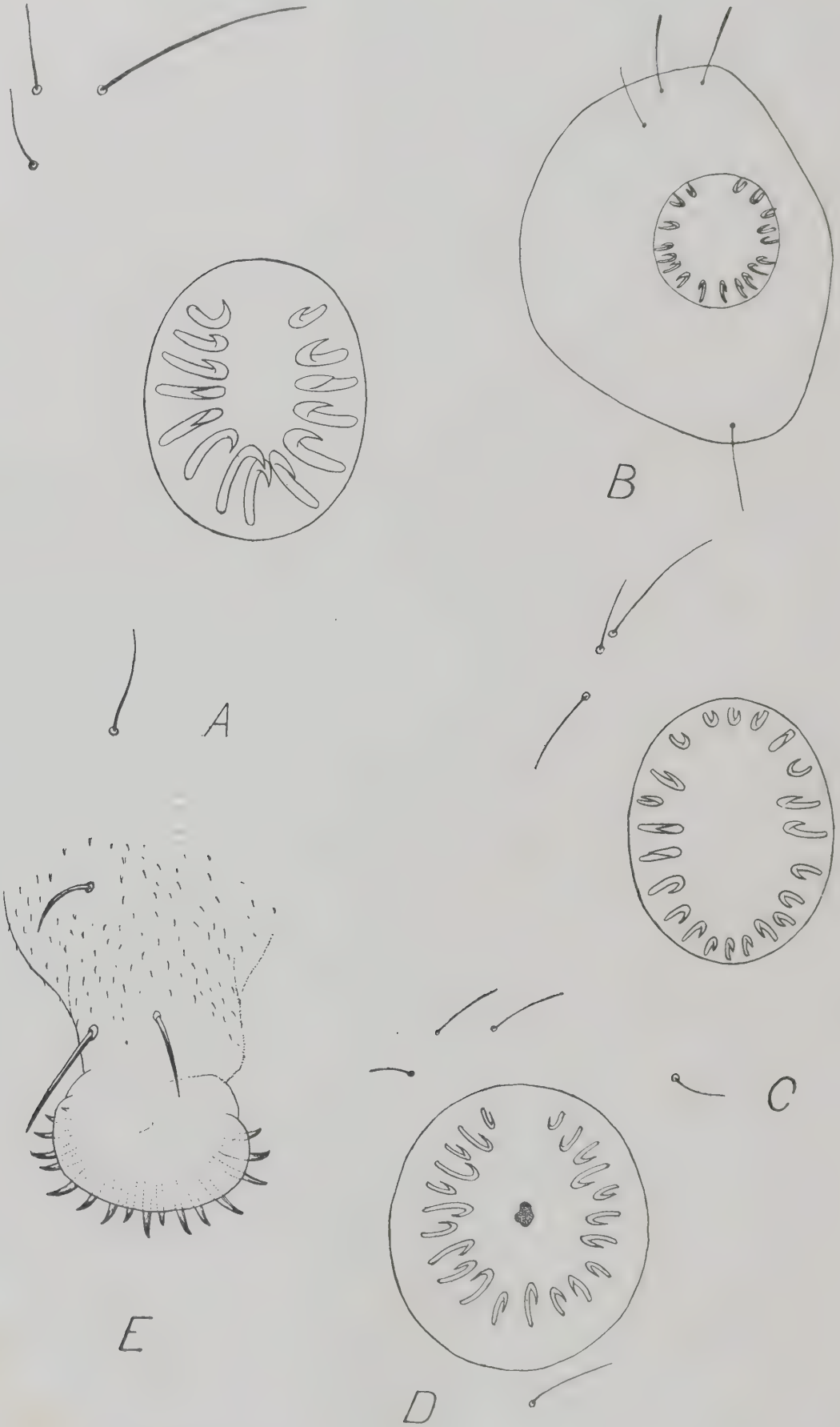

A
B
C
D
E

PLATE 106

Larval structures:

A.—*Pectinophora gossypiella:* Crochet arrangement of abdominal prolegs.
B.—*Crocidosema plebeiana:* Crochet arrangement of abdominal prolegs.
C.—*Pyroderces rileyi:* Crochet arrangement of abdominal prolegs.
D.—*Dicymolomia julianalis:* Crochet arrangement of abdominal proleg.
E.—*Heliothis obsoleta:* Crochet arrangement of abdominal proleg.

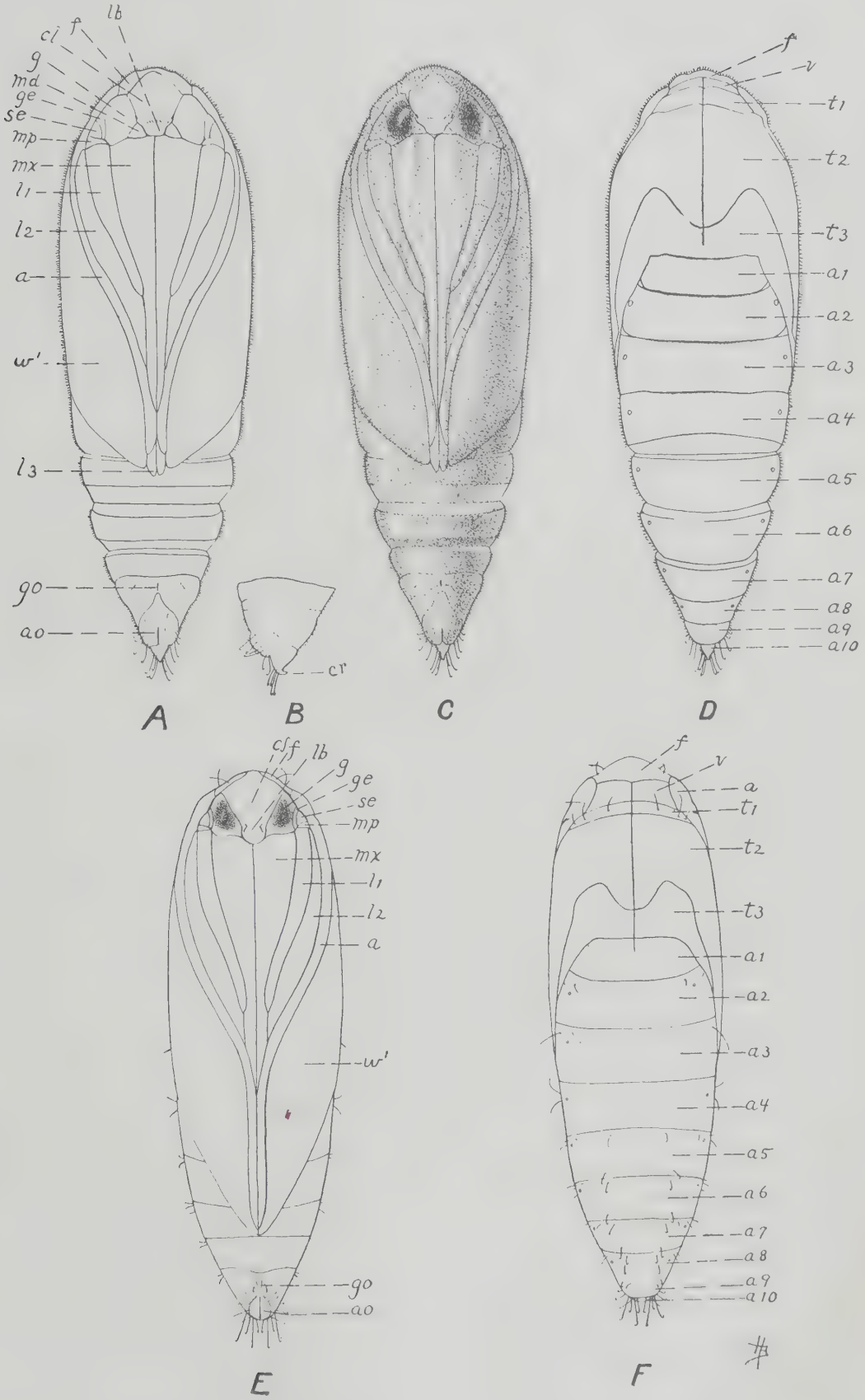
PLATE 107

Pupal structures:

A.—*Pectinophora gossypiella:* Ventral view of pupa.
B.—*Pectinophora gossypiella:* Caudal end of pupa, lateral view.
C.—*Pectinophora gossypiella:* Mature pupa, ventral view, shaded to show eyes of imago visible through pupal skin and characteristic pubescence of the pupa.
D.—*Pectinophora gossypiella:* Dorsal view of pupa.
E.—*Pyroderces rileyi:* Ventral view of pupa.
F.—*Pyroderces rileyi.* Dorsal view of pupa.

Explanation of symbols applied to pupæ on Plates 107–109.

a=antenna.
a^1 to a^{10}=abdominal segments 1 to 10.
ao=anal opening.
cl=clypeus.
cr=cremaster.
f=front.
f^1=femora of prothoracic leg.
fcs=fronto-clypeal suture.
g=gena.
ge=glazed eye.
go=genital opening.
lb=labrum.
l^1=prothoracic leg.
l^2=mesothoracic leg.
l^3=metathoracic leg.
lp=labial palpi.
md=mandible.
mp=maxillary palpus.
mx=maxilla.
pf=pilifer.
se=sculptured eyepiece.
t^1=prothorax.
t^2=mesothorax.
t^3=metathorax.
v=vertex.
w^1=mesothoracic wing.


lb
cl
f
g
md
ge
se
mp
mx
l1
l2
a
w'
l3
go
ao
A
cr
B
C
f
v
t1
t2
t3
a1
a2
a3
a4
a5
a6
a7
a8
a9
a10
D
cl f
lb
g
ge
se
mp
mx
l1
l2
a
w'
go
ao
E
f
v
a
t1
t2
t3
a1
a2
a3
a4
a5
a6
a7
a8
a9
a10
F

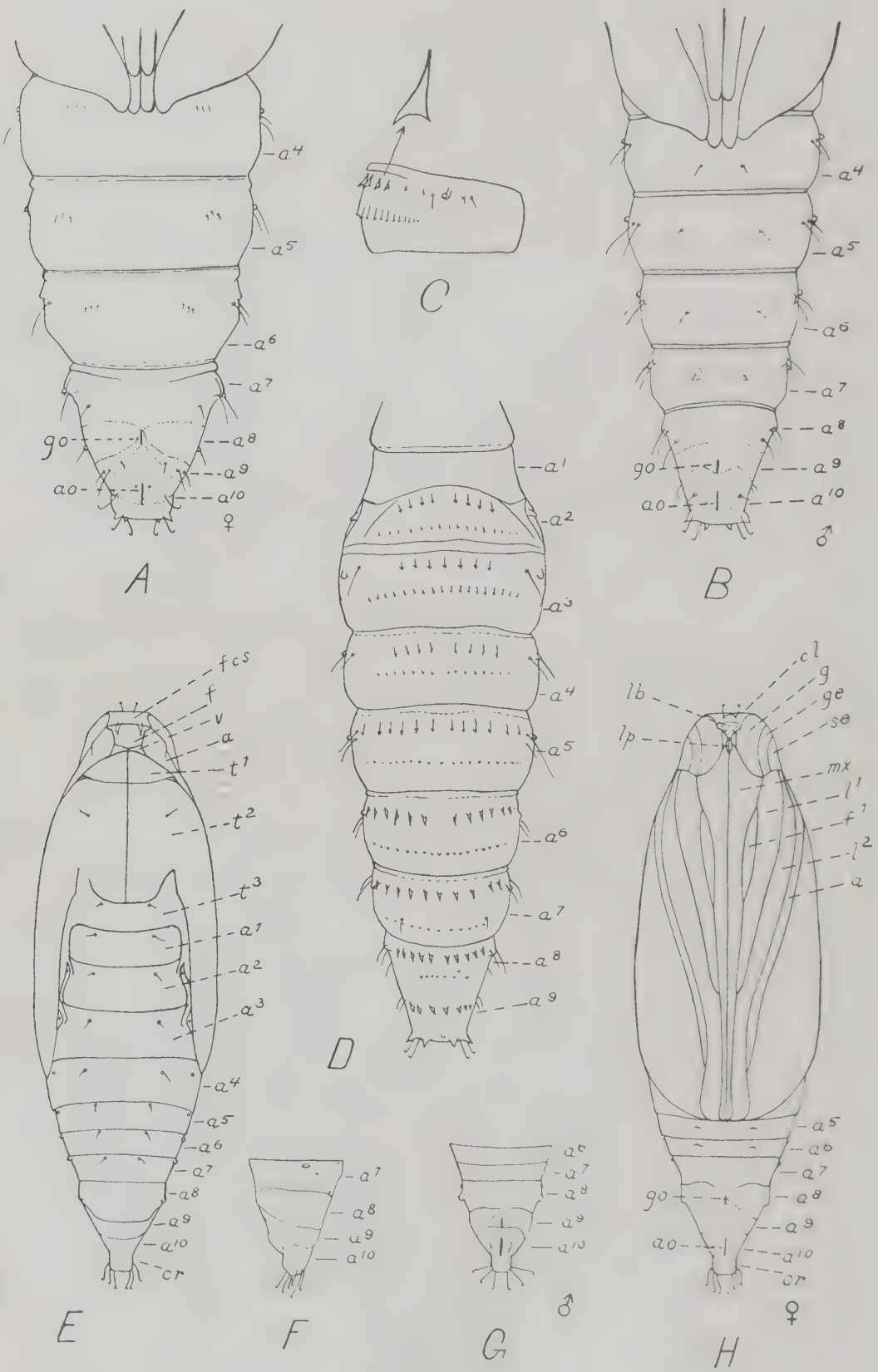

A
B
C
D
E
F
G
H
go
ao
♀
♂
cr

PLATE 108

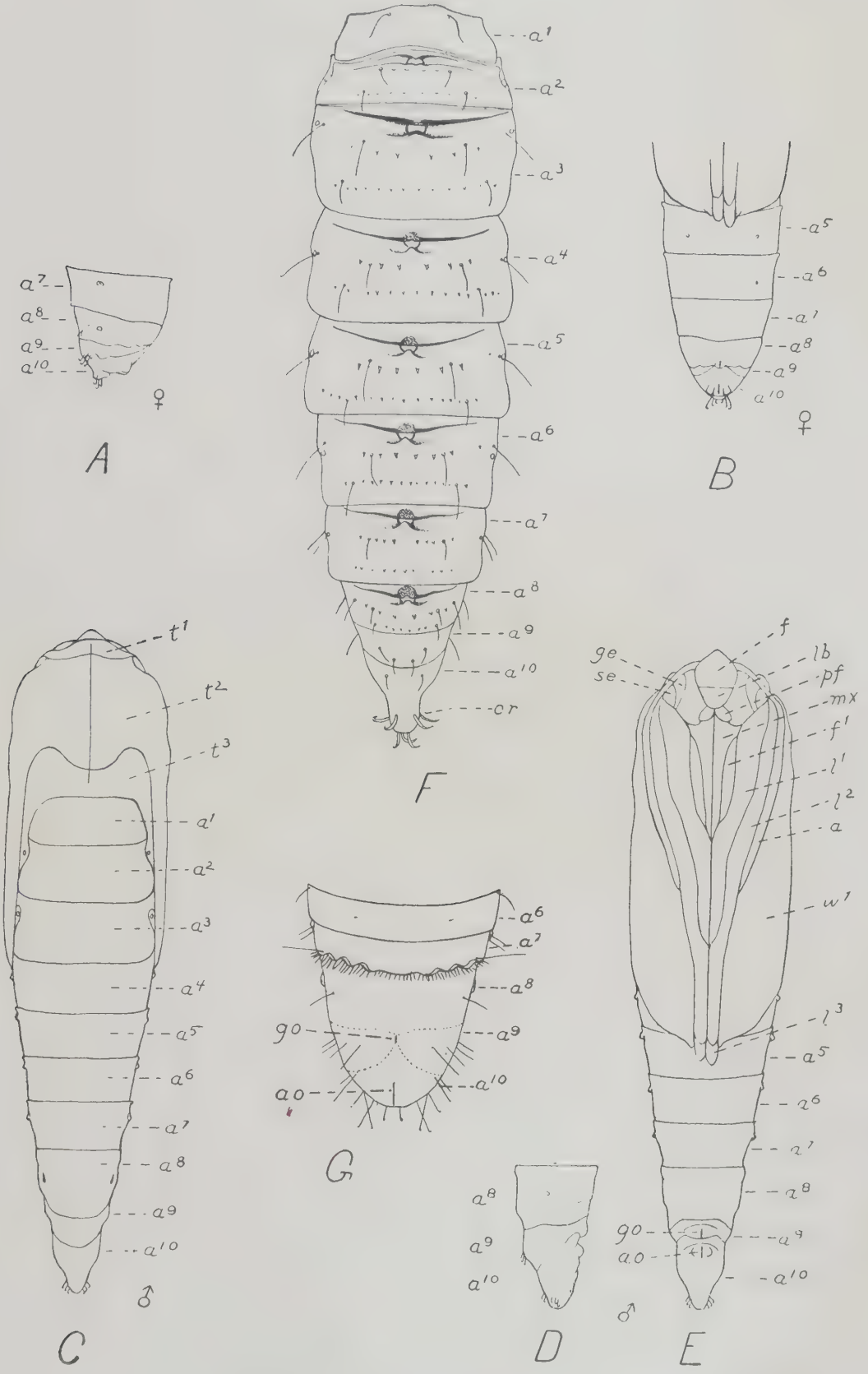
Pupal structures:

A.—*Crocidosema plebeiana:* Abdomen of female pupa, ventral view.
B.—*C. plebeiana:* Abdomen of male pupa, ventral view.
C.—*C. plebeiana:* Lateral view of an abdominal segment, showing arrangement and character of dorsal spines; one spine greatly enlarged to show shape.
D.—*C. plebeiana:* Abdomen of pupa, dorsal view.
E.—*Dicymolomia julianalis:* Dorsal view of pupa.
F.—*D. julianalis:* Caudal end of pupa, lateral view.
G.—*D. julianalis:* Caudal end of male pupa, ventral view.
H.—*D. julianalis:* Ventral view of female pupa.

PLATE 109

Pupal structures:

A.—*Meskea dyspteraria:* Caudal end of female pupa, lateral view.
B.—*M. dyspteraria:* Abdomen of female pupa, ventral view.
C.—*M. dyspteraria:* Male pupa, dorsal view.
D.—*M. dyspteraria:* Caudal end of male pupa, lateral view.
E.—*M. dyspteraria:* Male pupa, ventral view.
F.—*Amorbia emigratella:* Abdomen of pupa, dorsal view.
G.—*Telphusa mariona:* Caudal end of pupa, ventral view, showing peculiarly scalloped and fringed caudal margin of seventh abdominal segment.


a7
a8
a9
a10
♀
A
a1
a2
a3
a4
a5
a6
a7
a8
a9
a10
cr
F
a5
a6
a7
a8
a9
a10
♀
B
t1
t2
t3
a1
a2
a3
a4
a5
a6
a7
a8
a9
a10
♂
C
a6
a7
a8
a9
a10
go
ao
G
a8
a9
a10
D
ge
se
f
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pf
mx
f1
l1
l2
a
w1
l3
a5
a6
a7
a8
a9
a10
go
ao
♂
E

BIOLOGY OF THE SMARTWEED BORER, PYRAUSTA AINSLIEI HEINRICH[1]

By GEORGE G. AINSLIE, *Entomological Assistant*, and W. B. CARTWRIGHT, *Scientific Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

The attention of the senior author was first called to the smartweed borer in 1912, when hibernating larvæ were found in cornstalks at Franklin, Tenn. The economic status of this insect was undetermined at that time, but field and rearing records made in Tennessee and neighboring States since then have indicated that it is of no importance as a pest. At present, however, it is of considerable interest because of its similarity, both in habits and appearance, to the European corn borer (*Pyrausta nubilalis* Hübner). Until recently, also, it has been confused with another similar species, *P. penitalis* Grote, which feeds on lotus; and the purpose, in part, of this paper is to rectify this error.

Although Dr. E. Mosher (*7, p. 264*)[2] recorded differences of structure and the present authors found distinct variations in habit between the insect under discussion and the true *Pyrausta penitalis*, the former was first definitely recognized as an undescribed species by Mr. Carl Heinrich (*6*) of the Bureau of Entomology. Mr. Heinrich gives in detail the morphological characters separating the species *nubilalis*, *penitalis*, and *ainsliei* in all stages. Chittenden (*1*) has well summarized all the available records of the smartweed and lotus borers, although he was not aware that two species were included.

DISTRIBUTION AND HOST PLANTS

The smartweed borer is known to occur in Massachusetts, New York, Pennsylvania, Ohio, and Illinois; and the writers have taken it at numerous points in Tennessee and Kentucky and at Clemson College, S. C. *Polygonum pennsylvanicum*, its principal food plant, occurs throughout the eastern half of the United States, and it is likely that the distribution of the borer is coextensive therewith.

The plants in which the larvæ are found must be divided into two groups, namely, food plants proper and shelter plants.

[1] In recent papers by Flint and Malloch (*3, 4*), the name *Pyrausta obumbratalis* Lederer (misspelled *obumbratilis*) is used for this species. While it is possible that *ainsliei* will prove to be a synonym of *obumbratalis*, it seems inadvisable at this time to use this latter name for this species, for, until Lederer's type can be examined and its exact identity and relation to the other species under discussion determined, its use will simply add confusion to a matter which seems in a fair way to be solved.

[2] Reference is made by number (italic) to "Literature cited," p. 844.

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Washington, D. C.
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Key No. K-93

FOOD PLANTS

Riley (according to Chittenden, *1*, *p. 454*), who first noted what was probably this species, found larvæ in stems of *Polygonum incarnatum;* and Hart (*5*, *p. 182*) mentions that it has been reared from the same species at Urbana, Ill. Chittenden states that there is a moth in the National Museum reared from stems of *Polygonum hydropiperoides*. The foregoing references occur under the name of *Pyrausta penitalis*, but relate without doubt to *Pyrausta ainsliei*. After investigating the matter in New York, Dr. E. P. Felt writes that in his opinion—

> *Pyrausta ainsliei* occurs very commonly in *Polygonum pennsylvanicum* in this section [New York] and much more rarely in *P. lapathifolium*.

Mr. D. J. Caffrey writes that *Pyrausta ainsliei* has been reared from *Polygonum persicaria* in Massachusetts.

The work of the present authors indicates very clearly that south of the Ohio River, at least, *Pyrausta ainsliei* breeds only in *Polygonum pennsylvanicum*. Despite the most careful and persistent search they have failed to find either larvæ or eggs, or any trace of them, on plants of any other species even though growing in the immediate vicinity of *Polygonum pennsylvanicum* and often in the same clump. The species of the genus Polygonum are often confused, and determinations of plants for entomological purposes are so often made carelessly or from insufficient material that further work appears necessary in order that the occurrence of this borer in species other than *Polygonum pennsylvanicum* may be verified. As *Polygonum incarnatum* is now considered a synonym of *Polygonum lapathifolium* the following are here listed as reported natural food plants of *Pyrausta ainsliei: Polygonum pennsylvanicum*, *Polygonum lapathifolium*, *Polygonum hydropiperoides*, and *Polygonum persicaria*.

It should be stated that although never found on them in the field, larvæ have been reared from eggs to full-size caterpillars on leaves of curled dock (*Rumex crispus*) and buckwheat (*Fagopyrum fagopyrum*), both of which are close relatives of Polygonum. Leaves of all common weeds and plants were offered to the larvæ, but in every case except the two mentioned above they were either refused or only slightly gnawed. On leaves of lotus (*Nelumbo lutea*) the larvæ in several experiments starved to death after merely pitting the leaf surface. Mr. Heinrich's statement (*6*, *p. 175*) that we have reared these larvæ to maturity on *N. lutea* is an error.

SHELTER PLANTS

The other group, shelter plants, includes all plants the stems of which are entered by larvæ seeking winter quarters. The list of such plants will eventually contain practically all the pithy stemmed weeds and plants the bark of which is not too dense to permit the entrance of the larvæ. Some of the larvæ remain in the stems of smartweed, but for some

obscure reason many leave their food plant and seek entrance to anything that will give them dry quarters through the winter. The plants in which larvæ have been found by the authors are as follows: Corn (*Zea mays*), ragweeds (*Ambrosia trifida* and *Ambrosia artemisiaefolia*), cocklebur (*Xanthium communis*), goldenrod (*Solidago* spp.), aster (*Aster* spp.), timothy (*Phleum pratense*), cattail (*Typha latifolia*), beggartick (*Bidens bipinnata* and *B. frondosa*), and numerous other wild plant stems not in condition for determination. Dr. Felt adds *Brassica arvensis* and Chittenden (*1*) lists raspberry stems, to which the larvæ gained entrance through the cut ends. *Eupatorium* sp., in which larvæ were found in Missouri according to Chittenden, is also undoubtedly a shelter plant. Aside from *Polygonum* spp. the foregoing plants are in no sense food plants. The larvæ burrow the stems enough to construct a cavity sufficiently large to contain them; and even in this process, as the authors have observed, they do not swallow the plant tissue but eject it from the mouth. It is this habit of seeking shelter wherever it may be found, especially in cornstalks, that seems likely to lead to some confusion, for the larvæ are so similar to those of *Pyrausta nubilalis*, the European corn borer, that without careful laboratory study the two can not be differentiated.

SEASONAL HISTORY AND HABITS

In Tennessee there are two generations of the smartweed borer each year. Adults reared at Knoxville emerged from May 26 to October 30 with two well-defined periods of maximum abundance, the first from June 20 to July 5 and the second from August 18 to 30. Moths emerging in June at once oviposit, and the resulting larvæ complete their growth early in August and immediately pupate in their larval burrows in the smartweed stems. The moths emerge later in the same month and give rise to the second generation of larvæ, which reach full growth before winter and without further feeding remain in the food or shelter plants unchanged until they pupate in May and June of the following year.

Very few published data are available. Hart (*5, p. 182*) states that moths (probably of this species, as there is no Nelumbo near Urbana) were taken at light at Urbana, Ill., from May 19 to August 6, and that a single moth was reared July 1. In Missouri moths issued from smartweed from May 29 to June 6, and others are labeled October 9. Although scattering data on this species are included in his paper, Chittenden's conclusions do not agree with the actual life history as the authors have found it, and his statements must be taken, in the main, to apply to *Pyrausta penitalis*.

In a reared series of larvæ from eggs hatching August 16 a number of moths emerged October 13 and 15. This is difficult to explain except on the ground of abnormal conditions, for it does not seem possible that in nature moths emerging so late could produce another generation, and

under natural conditions neither pupæ nor moths have been found at this time of the year.

HABITS OF THE MOTHS

The moths frequent low, moist situations where the food plants grow normally. During the day they rest on or under the leaves and when disturbed make low direct or circuitous flights within the bounds of their haunts.

THE EGGS

Eggs have been taken many times in the field, but oviposition has not been observed. It doubtless occurs at dusk or during the night, and possibly on cloudy days, as the moths seem active only at such times. The eggs are laid in small patches or often in rows, with the individual eggs overlapping shingle fashion, on the underside of the leaves, more often those near the tips of the branches, and either on the leaf blade proper or close beside the midrib in the angle between it and the blade.

Near Union City, Tenn., on August 8, 1919, the senior author found an isolated clump of six plants of *Polygonum pennsylvanicum*. Thirty egg masses were found on these plants, all but one or two close beside the midrib on the under surface of the leaf. In three instances there were more than one mass on a leaf, but the difference in the stage of development clearly showed that they had been laid at different times. The number of eggs per mass varied from 4 to 16, the average being 9.3. In another collection of 17 egg masses made at Knoxville, August 12, the number of eggs varied from 7 to 14, with an average of 9.47 per mass.

As the egg has not heretofore been described, its description follows:

EGG.—Flat, thin, scalelike, laid in flat masses or rows of from 4 to 16, shingle fashion, each egg about half overlapping its predecessor. The individual egg is broadly elliptic, sometimes almost circular in outline, about 1.213 mm. long and 0.886 mm. broad. Chorion evenly reticulated all over with a close network of very fine but sharply elevated lines. Pale watery-greenish in color, nearly transparent when first laid, soon becoming more opaque, after which the embryo takes shape as a darker green, more transparent object in the center. No marked change then occurs until just before hatching, when the eyes and the mandibles darken, the color spreading to the whole head which becomes brown and plainly visible and appears detached because of the paleness and practical invisibility of the larval body which lies bent around the periphery.

The period of incubation in June and July is six days, in late August five days.

HABITS OF THE LARVÆ

Upon hatching, the young larvæ at once enter the stem near the tip of a branch, choosing the base of a petiole for their point of attack. That they are somewhat gregarious at this stage is shown by the fact that all the larvæ hatching from one egg mass usually enter the stem at the same point, which may be several inches from the egg mass. Thus in the first

and second instars burrows are often found containing a number of larvæ. Their work very quickly results in the wilting of the tender tips above the point of attack, and these drooping tips soon become to the observer an almost certain indication of the presence of the young larvæ. As soon as the food supply here is exhausted the larvæ desert this portion of the stem and scatter, each reentering at another point to make a burrow of its own, and thereafter only one larva is found in a burrow, although it often happens in a thickly infested stem that these burrows are practically continuous. The stems of *Polygonum pennsylvanicum* are thick-walled and succulent, with only a very small central cavity. The larvæ cut into this cavity, almost invariably entering at the swollen node just below the base of the ocrea, and consume the succulent tissue, leaving only the very thin, fibrous, outer bark. They do not hesitate to abandon a burrow and seek another location whenever the food supply fails. The larger stems are attacked first, but later the branches are utilized, often those so small that the larvæ can scarcely crowd into them. The burrows are kept clean, all excrement being disposed of through the entrance, which is left open, although with the growth of the plant it often partially heals.

Larvæ of the first generation make no effort to leave the smartweed stems but pupate in them as soon as fully fed. Those of the second generation attack the plants in the same way and feed as did their progenitors until they are fully grown. This stage is reached about the last of August, and thereupon many of the larvæ abandon their host plant and seek shelter elsewhere. Those entering cornstalks have been particularly noted. Neither thoroughly dry nor green stalks suit them as well as those of intermediate condition. They enter preferably under a leaf sheath or behind an ear. Their presence is indicated by the fluffy white pith showered from the entrance hole upon the leaves below. The entrance hole is perfectly round and clean-cut, and the burrow within is of equal diameter, 3 to 3.5 mm., and is kept clean and free of all cuttings and excrement. It turns downward from the entrance and is from 1 to 4 inches long. Early in October the larva closes the entrance with a drum-tight sheet of silk, quite effectively camouflaged by the incorporation of a few brownish particles of the chewed bark.

As far as determined the larvæ are not torpid during the period of hibernation. Repeated collections of larvæ in the field during the winter show them always quick to respond when disturbed. There is no evidence that they consume any food before pupation after leaving their food plants in the fall. In making their winter burrows in the shelter plants they do not swallow the tissue but discharge it from the mouth in sawdust-like particles.

No very definite cocoon is constructed by either generation. In smartweed the burrow is lightly plugged above and below the pupa

with pith particles interwoven with silk, and sometimes in the larger cavities a light cocoon is constructed, hardly more than a network of silk fibers. The burrow formed in smartweed by the larvæ of the first generation runs upward from the entrance; and the pupal chamber, in which the pupa lies head downward, is 1 or 2 inches above the exit hole. The emerging moth breaks the partition and leaves the pupal envelope in the chamber. In corn the cocoon is even less elaborate, and the most evident difference is that the pupa lies head upward in the burrow.

REARING RECORDS

Eggs were easily obtained from moths collected in the field and confined in lantern-chimney cages with a potted smartweed or in 1-ounce tin boxes containing a leaf of the same plant. The eggs hatched normally, and the young larvæ were transferred singly to 1-ounce tin boxes for rearing. The larvæ while young thrived on smartweed leaves, but in later stages they preferred the stems.

Table I contains the condensed data obtained from a series of larvæ hatching from eggs laid July 21 and from miscellaneous rearings from partly grown larvæ taken in the field.

TABLE I.—*Length, in days, of instars and stages of Pyrausta ainsliei*

Stage.	Maximum.	Minimum.	Average.	Number averaged.
Egg	6	6	6	.....
Larva:				
Instar I	4	3	3.27	15
II	6	3	4.64	14
III	7	4	5.22	14
IV	7	4	5.55	9
V	12	3	7.66	6
VI	28	18	23.67	3
Pupa	16	12	13.33	3
Total	.....	.....	69.34	.....

Table II contains similar data obtained from a series of 60 larvæ reared individually from eggs hatching August 18.

TABLE II.—*Length, in days, of instars and stages of Pyrausta ainsliei*

Stage.	Maximum.	Minimum.	Average.	Number averaged.
Egg	5	5	5	.....
Larva:				
Instar I	2	2	2	60
II	8	3	4.90	50
III	9	3	5.30	43
IV	18	5	10.31	32
V	37	20	24.66	12
Pupa	17	6	12.20	5
Total	.....	.....	64.37	.....

It will be noted that in Table II only five instars are listed, but that the fourth is nearly equal to the combined length of the fourth and fifth of Table I. It is possible that an error has been made here, but the notes are clear. The matter will be reviewed another year.

Chittenden mentions 11 and 17 days, respectively, as the lengths of the pupal stage of two specimens reared by him from cornstalks from Kansas.

NATURAL CONTROL

The smartweed borer varies greatly in abundance from year to year, and this seems to be due, in Tennessee at least, to variation in the abundance of its parasites. Here the most important of these appears to be (*Panzeria*) *Pyraustomyia penitalis* Coq., as over 40 per cent of the larvæ taken in the field at Knoxville for rearing were killed by it. Chittenden notes that this same species also killed more than 50 per cent of the larvæ taken by him in raspberry stems. The host grows normally and reaches its final instar before the maggot emerges. In its last instar the host becomes sickly and inactive, paler than normal, and finally incloses itself in a loose webbing. The parasite maggot emerges and pupates beside or partly within the remains of its host, often closely crowded into the cavity with them. In the overwintering larvæ the parasite remains within its host's body until spring and about the middle of May emerges and pupates in the normal manner. The pupal period for the fly varies from 13 to 16 days, being more often the latter. The flies that have been reared by the authors have emerged during two distinct periods—May 30 to June 10 and August 18 to September 12—coinciding closely with the normal dates for the emergence of the moths. This leads to the assumption that the flies must attack the host larvæ during their early instars.

Coquillett (*2, p. 15, 17, 19, 27*) records three other tachinid flies (two of them quoted from Townsend (*9, p. 467*)) as reared from "*Pyrausta penitalis*"—namely, *Exorista vulgaris* Fall., *Hypostena variabilis* Coq., and *Phorocera comstocki* Will.; but the information given is not sufficient to determine whether they are parasites of *Pyrausta ainsliei* or of the true *Pyrausta penitalis*.

Cremastus facilis (Cresson) was reared by Chittenden.

Three apparently distinct hymenopterous parasites have been found by the writers. One of these had spun a white cocoon and attached it to the remains of a host larva in its burrow. The second species was represented by small grubs which filled a dead larva. These grubs later made gray cocoons, only one of which developed. Two grubs of the third species were found attached externally to a larva. One of the grubs developed to an adult and was determined by Gahan as *Microbracon* sp., a male, and not specifically determinable. The authors have not received determinations of the other material.

Aside from true parasites, a coleopterous larva found preying on a larva of *Pyrausta ainsliei* was reared and determined by Schwarz as *Callida decora* Fab. Larvæ of *Chauliognathus pennsylvanicus* DeGeer are often found in the burrows and doubtless make way with some of the borers. In two instances they have been found feeding upon the contents of the puparia in the stems. Forficulids have been found in the burrows, but they probably act merely as scavengers.

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EFFECTS OF X-RAYS ON TRICHINÆ

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INTRODUCTION

The object of the experiments that are described in this paper was to determine whether X-rays exert deleterious influences on trichinæ (*Trichinella spiralis*), with a view to the practical application of X-ray radiation to the destruction of trichinæ in pork. These experiments were performed with the cooperation of a commercial firm that was operating X-ray machines in Florida. The experiments were planned and the details arranged by B. H. Ransom, Chief of the Zoological Division of the Bureau of Animal Industry, in consultation with the roentgenologist of the firm in question. The former supervised the tests made by the writer to determine the effects of the X-ray treatment upon the trichinæ, while the latter carried out the portions of the investigations relating to the X-ray treatment, calculations of the X-ray dosages used, etc.

The number of experiments that have been performed are insufficient to warrant any definite conclusions concerning the feasibility of applying X-ray radiation to the destruction of trichinæ in pork in routine packing-house procedure. Aside from the fact that the expense involved may render that procedure impracticable, much more experimental work than is presented in this paper would be required to demonstrate whether X-ray treatment could be depended upon to destroy trichinæ. The experimental data at hand are of interest from a general scientific viewpoint, however, and it is from that point of view that they are presented.

In a discussion of the effects of X-rays on the flour beetle (*Tribolium confusum*), Davey, [1] referring to his own work and the work of various other investigators, states:

> X-rays may act upon an organism (or on a single type of cell in that organism) in one of three ways: (1) to produce a stimulation; (2) to produce a destructive effect which takes place only after a certain latent interval; (3) to produce an instant destructive effect.

That the effects of X-rays on trichinæ so far as they are injurious become evident only after the parasites are subjected to influences that stimulate them to growth and development, or, in other words, after they reach the intestine of a host in which they normally attain sexual maturity,

[1] DAVEY, Wheeler P. THE EFFECT OF X-RAYS ON THE LENGTH OF LIFE OF TRIBOLIUM CONFUSUM. *In* Jour. Exp. Zool., v. 22, No. 3, p. 575–576. 1917.

and accordingly, that X-rays act on trichinæ in the second of the three ways mentioned above, is indicated by the results of the experiments recorded here.

METHODS OF EXPERIMENT

The trichinous meat used in these tests was obtained from hogs (series I, II, III, and V) and guinea pigs (series IV). The animals were artificially infected by feeding them trichinous pork. The infested pork to be exposed to X-rays was obtained from hogs that were killed several months after artificial infection. Trichinous guinea-pig meat was obtained from animals kept about a month after artificial infection.

Trichinous pork was packed in wooden or cardboard boxes in Washington, forwarded to Florida, where the exposure to X-rays was made, and promptly returned to Washington, where it was fed to experimental animals in order to determine the effects of the exposure. In a few cases portions of the meat that had been exposed to X-rays were digested in an acidified solution of scale pepsin, the decapsuled larvæ were examined, and the results were compared with those of the feeding experiments. Infested guinea pigs were shipped alive to Florida about 30 days after artificial infection. The animals were killed with chloroform in Florida, the skins and viscera were removed, and the carcasses were placed in boxes, exposed to X-rays, and returned to Washington.

The feeding experiments were performed in Washington. A quantity of the treated meat was ground up in a meat chopper and fed to a number of rats and, in some cases, mice. Unless they died as a result of infection with trichinæ the animals were killed at various intervals and examined for evidence of infestation with trichinæ as noted in connection with each experiment. Controls on the meat from the same lots as those exposed to X-rays showed that in all cases in which it was possible to maintain controls the untreated meat contained viable trichinæ capable of normal development. In those cases in which the entire carcasses of trichinous guinea pigs were exposed to X-ray treatment it was of course not possible to maintain controls.

EXPERIMENTS

SERIES I

X-RAY DOSAGE.—The units of dosage used in this series of experiments are described by the roentgenologist under date of January 20, 1917, on which day the exposures to X-rays were probably made,[1] as follows:

> I adopted a purely arbitrary unit, 1,000 of which units are equivalent to a dosage received at a distance of 5 inches from the focal spot of a Coolidge tube with a current of 4.2 milliamperes and a pressure of 70 kilovolts across the tube terminals. Treatment continued for 42 minutes. In ordinary X-ray terms this is equivalent to 172 milliampere minutes with a 6½-inch gap and a 5-inch distance.

[1] The meat was received in Washington on January 22, and feeding experiments were begun on January 23.

EXPERIMENT 1.—Strength of dosage, 2,899 units.

Twelve rats and two mice were fed in this experiment.

Rats 1, 2, and 3 were fed on January 23. Rats 1 and 2 were chloroformed on January 26. No trichinæ were found in the intestines. Rat 3 died on February 23; diaphragm negative.

Rats 4 to 9, inclusive, were fed on January 25. Rat 4 was killed on January 26. Trichinæ were found in the intestine. The parasites were about ready to molt. They were somewhat paler than normal. Rat 5 was killed on January 27. No trichinæ were found in the intestine. No. 6 was killed on February 26; diaphragm negative. No. 7 was killed on March 12; diaphragm negative. No. 8 and 9 were killed on March 15; diaphragms negative.

Rats 10 to 12, inclusive, were fed on January 30. Rats 10 and 11 were killed on January 31. A few trichinæ were found in the intestines of each animal. The parasites showed evidences of growth. Most of them were dead, however, having undergone granular degeneration.

Rat 12 was killed on February 1. A few trichinæ were found attached to the mucosa of the intestine. These showed evidence of growth.

Two mice were fed some of the treated meat on January 29. Mouse 1 was killed on January 30. No trichinæ were found in the intestines. Mouse 2 was killed on the same date. A few trichinæ were found in the intestines. The parasites were dead but showed evidence of growth. No details of structure were made out because the parasites had undergone granular degeneration.

EXPERIMENT 2.—Strength of dosage, 966 units.

Nine rats were fed in this experiment. Rats 1 to 3 were fed on January 23. Rat 1 was killed on January 26. No trichinæ were found in the intestines. Rat 2 was killed on February 2. A few trichinæ, apparently fully grown, were found in the intestines. The parasites showed rather striking malformations, which were especially pronounced in the reproductive organs. The gonads were shrunken. The uterus of female specimens contained eggs, but the latter were full of minute granules. The receptaculum seminis, which in normal females is crowded with spermatozoa, was empty.

Rat 3 was killed on February 6. No trichinæ were found in the intestines.

Rats 4 to 9 were fed on January 25. Rat 4 was killed on January 27; intestines negative. Rat 5 was killed on February 1; a few trichinæ were found in the intestines. The parasites showed marked evidence of degeneration. The cuticle was wrinkled; internally numerous vacuoles were seen; the sex cells appeared undeveloped; the worms showed very feeble movements. No. 6 was killed on February 26; diaphragm negative. No. 8 and 9 were killed on March 15; diaphragms negative.

EXPERIMENT 3.—Strength of dosage, 191 units.

Six rats were fed in this experiment. Three rats were fed on January 25. Rat 1 was killed on January 26. Trichinæ were found in the intestine. The parasites appeared normal as to size and structure. Rat 2 was killed on January 29; trichinæ in intestines normal; uterus of females packed with embryos. No. 3 was killed on February 2. Numerous trichinæ were found in the intestines; apparently normal.

Three rats were fed on January 26. One rat died on February 6. Numerous larvæ were found in the fluid expressed from the diaphragm. Intestines showed numerous trichinæ. The second rat was killed on February 12. Numerous unencysted larvæ were found in the diaphragm. The third rat died on February 24. Numerous encysted trichinæ were found in the diaphragm.

EXPERIMENT 4.—Strength of dosage, 81 units.

Five rats were fed in this experiment. Rats were fed on January 23. Rat 1 was killed on January 26; numerous live trichinæ were found in the intestines. Rat 2 was killed on January 29; intestines negative. Rat 3 was killed on February 3; numerous live trichinæ in intestines. Rats 4 and 5 were killed on March 15; diaphragms heavily infested with trichinæ.

EXPERIMENT 5.—Strength of dosage, 35 units.

Five rats were fed in this experiment. Rats 1 and 2 were fed on January 23. Rat 1 was killed on January 26; numerous live trichinæ in intestines. Rat 2 was killed on January 27; results as in No. 1. Rats 3 to 5 were fed on January 29. Rat 3 died on February 5; numerous live trichinæ in intestines. Rat 4 died on February 24; diaphragm heavily infested with trichinæ. Rat 5 died March 2; results as in No. 4.

EXPERIMENT 6.—Dosage, 19 units.

Three rats were fed on January 23 with the meat treated in this experiment. Rat 1 was killed on January 26; intestines contained many live trichinæ. Rat 2 died on February 12; diaphragm not infested. Rat 3 died February 26; diaphragm heavily infested.

ARTIFICIAL DIGESTION TESTS IN EXPERIMENTS 1 TO 6.—Some of the meat used in each experiment was digested in an artificial gastric juice January 23. The trichinæ thus freed from their capsules were examined microscopically. They showed no visible evidence of injury, being active under heat stimulation and remaining tightly coiled at room temperature and thus behaving like normal trichinæ.

RESULTS OF EXPERIMENTS OF SERIES I.—These experiments indicate that trichinæ are seriously injured by sufficiently high dosages of X-rays. Although the trichinæ in all six experiments when freed from their cysts by artificial digestion showed no apparent evidence of having been affected by the X-ray treatment, the parasites in the meat that had been exposed to the heaviest dosage (experiments 1 and 2) failed to complete their development when fed to experimental animals. Instead of growing and developing in a normal manner, after the molt

that regularly occurs soon after the parasites reach the intestines, they underwent degenerative changes, and even in those cases in which the parasites developed to sexual maturity the reproductive processes were seriously disturbed. That the reproductive organs are especially susceptible to X-ray injury is clearly shown by the results of experiment 2. In this experiment the larvæ succeeded in attaining maturity, but the sex cells evidently failed to function.

It is also interesting to note that despite the fact that several rats in experiment 3 were not fed until 6 days after the meat had been exposed to X-rays, the animals developed an infection. Thus, in this experiment there was evident neither an immediate nor a delayed effect of the X-ray treatment upon the encysted parasites.

SERIES II

Three experiments are included in this series. The units of dosage used in this series have the same relative values as those in series I. Under date of February 5, 1917, the roentgenologist writes as follows:

> The package marked "A" (experiment 7) was given 600 units, the package marked "B" (experiment 8) 300 units, and the package marked "C" (experiment 9) 350 units. The 300 units given to package "B" were given with low density and extra long time. The packages marked "A" and "C" were given the 600 and 350 units, respectively, at high tension—that is, close to the tube and with short time.

EXPERIMENT 7.—Strength of dosage, 600 units. The meat was exposed 19 minutes.

Three rats were fed on February 8. Rat 1 died on March 1; diaphragm negative. Rat 2 died on March 2; diaphragm showed a slight infestation with trichinæ. Rat 3 died on March 6; diaphragm slightly infested with trichinæ.

EXPERIMENT 8.—Strength of dosage, 300 units. The meat was exposed 46 minutes.

Three rats were fed on February 8. Rats 1 and 2 died February 12; live trichinæ were found in the intestines. Rat 3 died on February 26; numerous larvæ were found in the fluid expressed from the diaphragm.

EXPERIMENT 9.—Strength of dosage, 350 units. The meat was exposed 10½ minutes.

Four rats were fed on February 8. Rat 1 died on February 21; numerous live trichinæ in intestines. Rat 2 died on February 28; diaphragm infested with encysted trichinæ. Rat 3 died on March 1; results as in No. 2. Rat 4 died on March 2; diaphragm heavily infested with trichinæ.

RESULTS OF EXPERIMENTS OF SERIES II.—The parasites in the meat used in experiment 7 were evidently affected by the exposure. That some of them, however, escaped the injurious influences of the exposure to X-rays may be concluded from the results of the feeding experiments which resulted in rather slight infections.

SERIES III

In this series, which includes 12 experiments, the dosages used had the same relative values as those of the preceding series. The time of exposure and distance from the focal spot in the X-ray treatment of the various samples of meat in this series of experiments were not given in concrete terms, but in experiments designated by the letter A the meat was placed at twice the distance from the focal spot and held four times as long as in experiments designated by the letter B.

Two rats were used in each feeding experiment. The rats were fed on May 14.

EXPERIMENT 10A.—Dosage, 674 units.

Both rats were killed on June 15; diaphragms heavily infested with trichinæ.

EXPERIMENT 10B.—Dosage, 674 units.

Rat 1 died on May 29; intestines negative; diaphragm negative. Rat 2 died on June 12; diaphragm negative.

EXPERIMENT 11A.—Dosage, 924 units.

Rat 1 died May 28; intestine negative; diaphragm negative. Rat 2 died on June 12; diaphragm negative.

EXPERIMENT 11B.—Dosage, 924 units.

Both rats killed on June 15; diaphragms heavily infested.

EXPERIMENT 12A.—Dosage, 1,363 units.

The rats were killed on June 15; diaphragms negative.

EXPERIMENT 12B.—Dosage, 1,363 units.

The rats died on June 17; diaphragms negative.

EXPERIMENT 13A.—Dosage, 2,162 units.

The rats were killed on June 15; diaphragms negative.

EXPERIMENT 13B.—Dosage, 2,162 units.

The rats were killed June 15; diaphragms negative.

EXPERIMENT 14A.—Dosage, 1,081 units.

Rat 1 dead June 5; one unencysted larva found in diaphragm. Rat 2 killed June 15; diaphragm heavily infested.

EXPERIMENT 14B.—Dosage, 1,081 units.

Rat 1 dead June 12; diaphragm heavily infested. Rat 2 killed June 15; results as in No. 1.

EXPERIMENT 15A.—Dosage, 3,094 units.

Rats killed June 15; diaphragms negative.

EXPERIMENT 15B.—Dosage, 3,094 units.

Rats killed June 15; diaphragms negative.

RESULTS OF EXPERIMENTS OF SERIES III.—In this series of experiments trichinous meat subjected to dosages up to 1,081 units proved to be infective, whereas in experiment 2 (series I) a dosage of 966 units impaired the vitality of the reproductive cells of the parasites. Whether this can be accounted for on the basis of variation of trichinæ to the effects of X-rays or whether other factors were involved can not be stated.

SERIES IV

Under date of June 28, the roentgenologist states that the meat used in this series of experiments was—

exposed to the direct action of the rays at a distance of very nearly 25 cm. from the focal spot of a Coolidge-type tube. The pressure across the tube terminals was 73 kilovolts, measured by standard sphere gap, and also by ratios. The current through the tube varied during the time of treatment, which extended over a period of about 3 hours. The lowest reading was 4.2 milliamperes, the highest 4.9. This high reading, however, was for only a short time after the tube was started. The current gradually dropped during 10 minutes to 4.3 milliamperes, and during the rest of the treatment fluctuated between 4.2 and 4.3 milliamperes.

The boxes were so placed that the rays from other tubes in the machine had very little influence on the contents. By calculation it shows as negligible.

Box A was given an exposure of 42 minutes; box B an exposure of 84 minutes; box C an exposure of 126 minutes; and box D an exposure of 168 minutes. Following the system of measurement used by Davey,[1] which has the merit of being a complete expression of X-ray quantity, these dosages would read:

Box A $180\frac{MAM}{25^2}$ at 73 KV.

Box B $361\ \frac{MAM}{25^2}$ at 73 KV.

Box C $542\ \frac{MAM}{25^2}$ at 73 KV.

Box D $722\ \frac{MAM}{25^2}$ at 73 KV.

The rats used in this series of experiments were fed on July 31 and August 3. Five rats were fed in each experiment.

EXPERIMENT 16 (BOX A), 42 MINUTES.—Rats 1 and 2 died August 4. A few trichinæ were found in the intestines. The parasites showed evidence of growth. The sex cells were strikingly disorganized. Other organs also showed evidence of injury. Rat 3 was killed on August 20; diaphragm moderately infested. Rat 4 died on August 29; diaphragm moderately infested. Rat 5 died on September 16; diaphragm moderately infested.

EXPERIMENT 17 (BOX B), 84 MINUTES.—Rat 1 died on August 6; intestines negative. Rat 2 died on August 17; intestines and diaphragm negative. Rat 3 died on August 18; results same as in rat 2. Rat 4 died on August 20; results same as in rat 2. Rat 5 was killed on September 10; diaphragm negative.

EXPERIMENT 18 (BOX C), 126 MINUTES.—Rats 1 and 2 were killed on August 20; diaphragms negative. Rats 3 and 4 were killed on September 10; diaphragms negative. Rat 5 was killed on September 10; diaphragm slightly infested.

[1] Davey (OP. CIT., p. 586) states: "The voltage and distance are given directly and the product of the current and time is given, thus, '100 milliampere-minutes at 25 cm. distance at 50 kilovolts.' This is usually contracted to read $100\frac{MAM}{25^2}$ at 50 kv. It will be noticed that distance is expressed in terms of its square. This is because the intensity of X-rays varies inversely as the square of the distance."

EXPERIMENT 19 (BOX D), 168 MINUTES.—Rat 1 was killed on August 7; intestine negative. Rat 2 was killed on August 20; intestine negative and diaphragm negative. Rat 3 died on September 5; diaphragm negative. Rats 4 and 5 were killed on September 10; diaphragms negative.

RESULTS OF EXPERIMENTS OF SERIES IV.—The X-ray dosages used in these experiments were clearly injurious to the trichinæ. The smallest dosage used (experiment 16) had some effect, though it did not destroy the reproductive functions of all the parasites. In the three other experiments in which considerably larger dosages were used only 1 infection occurred among the 15 experimental animals on which the infectiousness of the meat was tested, and that infection was slight.

SERIES V

This series included six experiments. The dosages used in these experiments were not indicated, except that two samples were given similar dosages and that the remaining samples received graded dosages. Furthermore, the samples were mixed so that it is not known which samples received the larger or the smaller dosages. The samples were treated on March 24. Experimental rats were fed in Washington on March 27.

EXPERIMENT 20.—Rat 1 died on April 5; no trichinæ were found in the intestines. Rat 2 was killed on April 9; intestines contained live trichinæ; female trichinæ contained many embryos; diaphragm negative. Rat 3 was killed April 16; intestines positive; diaphragm positive. Rat 4 was killed on April 24; diaphragm heavily infested.

EXPERIMENT 21.—Rat 1 was killed on April 9; intestines contained live trichinæ, normal in appearance; female trichinæ contained eggs and embryos. Rat 2 was killed on April 16; intestines contained many live trichinæ. Rat 3 died on April 24; diaphragm heavily infested.

EXPERIMENT 22.—Rat 1 was killed on April 8; intestines negative; diaphragm negative. Rat 2 was killed on April 16; diaphragm negative. Rat 3 died on April 17; diaphragm negative. Rat 4 died on April 24; diaphragm heavily infested.

EXPERIMENT 23.—Rat 1 was killed on April 9; live trichinæ were found in the intestines; sex cells were atrophied; no larvæ were found in the diaphragm. Rat 2 was killed on April 16; no trichinæ were found in the intestines; diaphragm negative. Rat 3 was killed on April 23; diaphragm negative. Rat 4 was killed on April 23; one encysted larva was found in the diaphragm.

EXPERIMENT 24.—Rat 1 was killed on April 2; intestines contained numerous live and apparently normal trichinæ. Rat 2 was killed on April 8; live trichinæ were found in the intestines; diaphragm negative. Rat 3 was killed on April 16; intestines contained trichinæ, apparently dead; diaphragm negative. Rat 4 was killed on April 24; diaphragm heavily infested. Rat 5 was killed on April 24; diaphragm negative.

EXPERIMENT 25.—Rat 1 was killed on April 9; intestines contained live trichinæ; sex cells of trichinæ atrophied; diaphragm negative. Rat 2 was killed on April 16; diaphragm negative. Rat 3 died on April 19; diaphragm negative. Rats 4 and 5 were killed on April 24; diaphragm negative.

RESULTS OF EXPERIMENTS OF SERIES V.—The results of these experiments are in harmony with the results of the experiments recorded in the preceding pages. Trichinæ that showed sex-cell injuries (experiments 23 and 25) failed to produce a new generation. That a few larvæ in experiment 23 escaped injury is evident from the results of the feeding experiment with rat 4. It is interesting to note, however, that despite the fact that the parasites showed evidence of injury they were still alive on the fourteenth day after artificial infection. This indicates that X-rays exert a selective action on the sex cells of trichinæ and that injuries to the sex cells do not necessarily affect the other vital functions of the parasites.

DISCUSSION

The results of the experiments described in the foregoing pages show that trichinæ may be seriously injured by X-ray radiation. It is interesting to note that in experiments 1 to 6 inclusive (series I), larvæ isolated from the treated meat by artificial digestion appeared to be unaffected. These larvæ were normal as to color and general appearance, as viewed through the microscope and as indicated by their reactions to heat stimulation. The examination was made three days after treatment. The larvæ from the meat treated in experiments 1 and 2 (series I) were incapable, however, of attaining full sexual maturity in the intestines of rats or mice. Those in experiment 1 and some of those in experiment 2 underwent granular degeneration, while others in the latter experiment succeeded in attaining maturity without being capable of functioning sexually. The fact that no spermatozoa were found in the receptaculum seminis of the female indicates that successful copulation had not taken place.

It is also of interest to observe that a considerable degree of variation in resistance to X-rays is exhibited by trichinæ, since certain dosages proved to be destructive in some cases and not in others. This is possibly due, however, to other factors. It may be noted in this connection that trichinæ exhibit considerable variation in their resistance to cold [1] and in their resistance to heat.[2]

Assuming that a reliable and practically possible method of destroying the vitality of the sex cells in trichinæ by means of X-ray treatment of infested meat can be perfected, which is quite uncertain, it is still questionable whether such a method would be acceptable as a prophylactic

[1] RANSOM, B. H. EFFECTS OF REFRIGERATION UPON THE LARVÆ OF TRICHINELLA SPIRALIS. *In* Jour. Agr. Research, v. 5, no. 18, p. 819–854. 1916. Literature cited, p. 853–854.

[2] ——— and SCHWARTZ, Benjamin. EFFECTS OF HEAT ON TRICHINÆ. *In* Jour. Agr. Research, v. 17, no. 5, p. 201–221. 1919. Literature cited, p. 220–221.

measure, inasmuch as trichinæ are not inoffensive as intestinal parasites apart from the damage done by their migrating larvæ. Rats, for example, commonly die from intestinal trichinosis prior to the migration of the larvæ, and human beings also often suffer seriously from the effects of the intestinal stage of the parasites during the first few days after infection before the migrating larvæ have been produced. Consequently, unless the X-ray treatment has the effect of diminishing the injurious action of the intestinal stage of trichinæ upon the host as well as of destroying their powers of reproduction, it can scarcely be considered a satisfactory prophylactic measure. It is of interest to note in this connection that Tyzzer and Howell[1] found that encysted trichinæ that had been subjected to radium radiation failed to develop in mice. These investigators also determined that whereas radium radiation failed to destroy sexually mature trichinæ in live rats, trichinæ in rats which were radiated beginning with the second day after ingestion of trichinous meat showed retardation in development. Radiation of the larvæ in rats before they have begun to develop proved fatal to them.

SUMMARY

(1) Encysted trichinæ are injured by relatively heavy dosages of X-rays. So far as has been determined the injuries are not visible in the encysted or artificially decapsuled larvæ as structural or functional disturbances but become apparent only when the larvæ reach a suitable host animal in whose intestine they are normally capable of continuing their development.

(2) Trichinæ from meat that has been exposed to strong dosages of X-rays undergo rapid granular degeneration in the intestines of suitable hosts before they attain maturity.

(3) Encysted larvæ that have been exposed to lower but still injurious dosages of X-rays are able to continue development in the intestines of suitable hosts. Such larvæ, however, do not attain structural and functional sex maturity. The sex cells appear to be atrophied, and no evidence of successful copulation can be found. X-rays, therefore, appear to exert a more or less selective action on the gonads of trichinæ.

(4) Trichinæ appear to exhibit considerable variation in their susceptibility to X-rays, since certain dosages injured some parasites and failed to injure others. Whether the apparent variation in susceptibility of trichinæ to X-rays is an expression of an actual physiological variation or may be accounted for by other factors has not been determined.

(5) The experiments described in this paper do not warrant any definite conclusions as to the feasibility of using X-ray radiation as a practical means of destroying trichinæ in pork.

[1] Tyzzer, E. E., and Howell, James A. THE EFFECTS OF RADIATION ON THE DEVELOPMENT OF TRICHINELLA SPIRALIS WITH RESPECT TO ITS APPLICATION TO THE TREATMENT OF OTHER PARASITIC DISEASES. In Jour. Par., v. 3, no. 2, p. 43–56, 1 pl. 1916.

RELATION OF THE CALCIUM CONTENT OF SOME KANSAS SOILS TO THE SOIL REACTION AS DETERMINED BY THE ELECTROMETRIC TITRATION

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The importance of the soil reaction has led to the development of numerous methods for testing the neutrality, acidity, or alkalinity of the soil, and, if the soil is acid, for determining quantitatively the amount of agricultural lime necessary to add to the soil in order that it may have the reaction required for maximum crop production. No attempt will be made to review the literature on this subject, and only a few citations will be given.

Of the different tests designed simply to determine qualitatively the reaction of the soil, the litmus paper test is one of the oldest, best known, and probably most extensively used. This test has been subjected to much criticism, but this is probably due more to bad paper and faulty use than to intrinsic defects in the method. The official or Hopkins method (*15, p. 20*)[1] has been used for most of the acidity work done thus far on Kansas soils. It was found, however, that in some cases the indicated lime requirement appeared too low when studied in connection with the known cropping conditions of the soil. The well-known Veitch method (*14*) is probably the best quantitative measure of the lime requirements of the soil at the present time. There are several other methods proposed to determine the lime requirements of the soil, and each has its advocates. The strong advocate of any method is usually very free with his objections to some other method. All methods are limited in their application, and faults are often found with methods because the users extend the application further than the originators intended.

One difficulty in determining the soil reaction is to obtain the soil solution in the same concentration as it exists around the soil grains. Various methods have been proposed for securing this solution, but none have received general acceptance. Another factor is the facility with which the optimum reaction for best crop production is maintained in the soil. The concentration of the soil solution is in a state of continuous change. The film of water surrounding the soil grains in a soil of optimum water content tends to become saturated with the salts present in the soil. The

[1] Reference is made by number (italic) to "Literature cited," p. 867–868.

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addition of rain or irrigation water temporarily reduces the concentration. If some of the water is carried off in the drainage, it takes away a certain amount of the dissolved salts. At the present time calcium is removed from the soil more rapidly than any other base (*5, p. 23*). Lyon and Bizzell (*8*) found in lysimeter experiments that the equivalent of 485 pounds of calcium carbonate per annum leached from some soils. The continuous removal of calcium from the soil produces an unbalanced condition known as lime deficiency or acid soil. When calcium carbonate is added to the soil the balance is restored and the reaction is neutral or slightly alkaline.

The studies presented in this paper are not designed to settle any differences of opinion relative to the meaning of soil acidity nor to decide which is the best method of determining the lime requirement of the soil. They are presented as a contribution to the partial solution of a very complex as well as important problem. The electrometric titration has been used by a number of investigators (*6, 9, 10*). Because of its intrinsic value it was used in this study of the relation between the calcium content of some typical Kansas soil and the reaction.

MEANING OF SOIL ACIDITY

The following is usually taken as the meaning of acidity in soil: Total acidity means the total quantity of hydrogen ions which may be produced when the equilibrium is continually shifted by the introduction of hydroxyl ions. The quantity of hydrogen ions present at any one moment is regarded as the intensity of acidity. This definition would be inclusive and very convenient if it were not for the adsorptive power of colloids in soil. It will be shown that this intensity of acidity may be very small as related to the total acidity. Understood in this way, quantitatively, total acidity has the same meaning as potential acidity. Potential acidity may be due to undissolved substances, or to soluble compounds only partly hydrolyzed or dissociated. It appears also to be due to colloidal clay; but whatever it is due to, the conditions are such that as soon as more hydroxyl ions are introduced the equilibrium is shifted by the production of more hydrogen ions. The absolute neutral point obtains when the number of hydrogen ions and the number of hydroxyl ions are equal and each has a concentration of 10^{-7} per liter.

SOILS USED IN THIS STUDY

Twelve counties in Kansas have been surveyed and mapped by the Bureau of Soils, United States Department of Agriculture. Five of these counties were worked in cooperation with the Kansas Agricultural Experiment Station. These types have been sampled and analyzed by the Department of Chemistry, Kansas Agricultural Experiment Station (*1–4, 11*). Determinations have been made for nitrogen, phosphorus, potassium, carbon, carbon dioxid, and calcium. On the basis of these data

those soils which were thought most suitable were selected. In the description of these soils, the type names given by the Bureau of Soils are used. The soil numbers are those found in our soil series. The samples had been taken usually in three strata—namely, surface 0 to 7 inches, subsurface 7 to 20 inches, and subsoil 20 to 40 inches. For this work, surface soils mostly were selected, with a few accompanying subsoils. The soil number has a whole figure and a decimal. A surface soil is designated as 1083.1, subsurface as 1083.2, and a subsoil as 1083.3. Some of the samples were taken in only two strata.

The figures given for total calcium and carbon dioxid are taken from the publications to which reference has been made. In addition the authors have determined the calcium soluble in *N/1* hydrochloric acid and in *N/5* hydrochloric acid and the reaction as determined by the hydrogen electrode with accompanying titrations.

DETERMINATION OF ACID-SOLUBLE CALCIUM

Five gm. of soil were placed in 100 cc. *N/1* hydrochloric acid and shaken for one hour on a shaking machine, then placed in a thermostat at 40° C. and digested for 23 hours with occasional shakings. The acid-soluble calcium was determined by the volumetric permanganate method. The treatment with *N/5* hydrochloric acid was similar except that 10 gm. of soil and 200 cc. of the acid were used. The results on the calcium determinations are given in Table I.

TABLE I.—*Calcium and carbon dioxid in representative Kansas soils*

GROUP I, SOILS WHOSE INITIAL REACTION WAS MORE ALKALINE THAN IS INDICATED BY PH 8.3

Soil No.	County.	Soil type.	Total Ca.	Ca. soluble in *N/1* HCl.	Ca. soluble in *N/5* HCl.	CO_2.
			Per cent	*Per cent.*	*Per cent.*	*Per cent.*
1043	Russell	Benton loam	4.89	4.01	4.19	4.37
1227	Greenwood	Crawford clay	3.25	3.30	3.18	.84
1297	Montgomery	Oswego silt loams	1.41	1.14	1.03	.20
1199	Jewell	Laurel very fine sandy loam	1.11	.60	.53	.10
1206	do	Lincoln silty clay loam	1.01	.50	.69	.04
1186	Reno	Kirkland clay loam	.56	.48	.47	.10
1115	Finney	Richfield silt loam	.74	.32	.28	Trace.
1119	do	Pratt loamy fine sand	.60	.10	.09	Do.
1169	Reno	do	.40	.08	.05	.009

GROUP II, SOILS WHOSE INITIAL REACTION WAS BETWEEN PH 8.3 AND PH 7

1219	Greenwood	Osage loam	0.88	0.80	0.80	0.11
1047	Brown	Silt loam (bottom)	.80	.66	.57	.04
1039	Russell	Summit silty clay loam	.83	.62	.54	.16
1132	Shawnee	Osage silty clay loam	.87	.59	.54	Trace
1268	Montgomery	Osage clay (alfalfa land)	.79	.52	.49	None
1267	do	Osage silty clay loam	.76	.49	.38	Do.
1121	Finney	Finney clay	.78	.31	.30	Trace.
1131	Shawnee	Osage very fine sandy loam	.79	.28	.36	Do.
1070	Reno	Pratt loam	.42	.20	.14	Do.
1157	do	Arkansas clay loam	.70	.21	.19	.003
1120	Finney	Sandy loam	.44	.18	.16	Trace.
1072	Harper	Brown loam	.31	.18	.13	None.
1162	Reno	Pratt fine sandy loam	.49	.12	.12	.003
1160	do	Arkansas fine sand	.55	.08	.07	.002

TABLE I.—*Calcium and carbon dioxid in representative Kansas soils*—Continued

GROUP III, SOILS WHOSE INITIAL REACTION WAS MORE ACID THAN IS INDICATED BY PH 7

Soil No.	County.	Soil type.	Total Ca.	Ca. soluble in *N*/1 HCl.	Ca. soluble in *N*/5 HCl.	CO_2.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1141	Shawnee....	Summit silty clay loam	0.54	0.42	0.41	0.016
1136	.....do.....	Oswego silt loam	.55	.35	.31	.013
1285	Montgomery	Crawford loam	.61	.34	.32	None.
1287	.....do.....	Summit silty clay loam	.55	.28	.25	Do.
1058	Doniphan...	Brown silt loam	.47	.26	.21	Trace.
1284	Montgomery	Crawford loam	.57	.25	.24	None.
1190	Jewell.....	Jewell silt loam	.60	.24	.21	.01
1293	Montgomery	Bates stony loam	.38	.24	.29	None.
1191	Jewell.....	Colby silt loam	.63	.23	.21	.01
1135	Shawnee....	Crawford silty clay loam	.38	.23	.18	.01
1271	Montgomery	Bates loam	.40	.21	.19	None.
1143	Shawnee....	Boone fine sandy loam	.37	.20	.18	Trace.
1256	Greenwood..	.....do.....	.40	.18	.15	Do.
1262	Montgomery	Crawford loam	.39	.32	.....	None.
1233	Cherokee....	Oswego silty clay loam	.39	.17	.14	Trace.
1257	Greenwood..	Summit silty clay loam	.52	.17	.12	Do.
1265	Montgomery	Cherokee silt loam	.46	.16	.13	None.
1273	.....do.....	Bates very fine sandy loam	.32	.16	.14	Do.
1275	.....do.....	Oswego silt loam	.41	.15	.13	Do.
1232	Cherokee....	Bates silt loam	.29	.14	.10	Trace.
1277	Montgomery	Bates shale loam	.39	.14	.09	None.
1266	.....do.....	Bates very fine loam	.34	.11	.09	Do.
1239	Cherokee....	Summit silt loam	.51	.09	.08	Trace.
1243	.....do.....	Cherokee silt loam	.30	.09	.07	Do.
1280	Montgomery	Bates loam	.26	.09	.07	None.
1230	Cherokee....	Neosho silt loam	.35	.08	.07	Do.
1279	Montgomery	Bates very fine sand	.30	.06	.04	Do.
1148	Reno.....	Dune sand	.36	.05	.04	.00:
1244	Cherokee....	Bates fine sandy loam	.11	.05	.04	Trace.

DETERMINATION OF THE INITIAL REACTION AND THE ELECTROMETRIC TITRATION OF SOILS STUDIED

The apparatus used in these determinations was the same as that described in previous papers (*12*, *13*). Ten gm. of soil were weighed into a 250-cc. bottle which was used as the electrode vessel, and 100 cc. of carbon-dioxid free water were added. The bottle was closed with a large rubber stopper through which were inserted the hydrogen electrode and the capillary tube connecting with the calomel cell. The hydrogen after bubbling through the soil suspension passed through a water trap, and the tip of the burette used in the titration was inserted through a hole in this stopper. In this way contamination from the carbon dioxid in the air was prevented. These precautions are necessary, since these determinations require a number of hours.

The distilled water used in this work was freed from carbon dioxid by aeration. While water so treated is not as neutral as conductivity water, the purity was sufficient for these determinations. The reaction of various samples of this water ranged from $P_H 6$ to $P_H 6.6$. One-tenth cc. of *N*/10 alkali would change the concentration from about P_H 6 to P_H 8. The error due to the water is therefore small. After the apparatus was adjusted, the hydrogen gas was bubbled through until equilibrium was obtained. The time required for this depended somewhat on the character of the soil. During the entire time the electrode vessel was shaken about 60 times per minute by a shaking device. As soon as the readings on the millivoltmeter remained constant within a few millivolts for 15

minutes, the soil suspension was considered to be at equilibrium. This point was noted and taken as the initial reaction of the soil. A solution of saturated calcium hydroxid is very near *N/24*. For the sake of facility in making calculations this was made *N/25*. Since the final end product of calcium hydroxid or calcium oxid added to the soil is calcium carbonate, this equivalent is used in making the calculations. One cc. of *N/25* calcium hydroxid is equivalent to 0.002 gm. of calcium carbonate. One acre of soil 7 inches deep is assumed to weigh 2,000,000 pounds. Since 10 gm. of soil were used in a determination, the ratio of the calcium carbonate equivalent of 1 cc. of the calcium hydroxid is 1 : 5,000. Accordingly, each cubic centimeter of calcium hydroxid used to titrate is equivalent to 400 pounds of calcium carbonate per acre.

When the voltmeter reading at the initial equilibrium point had been obtained, the calcium hydroxid was added from the burette in small portions at a time until the equilibrium was again obtained at voltmeter reading equivalent to P_H 7. The total number of cubic centimeters used in the titration were recorded, and again small portions of calcium hydroxid were added till equilibrium was established at voltmeter reading equivalent to P_H 8.3. This is approximately the titration end point for phenolphthalein. Again the calcium hydroxid was added until equilibrium was established at reading equivalent to P_H 10. The latter point was somewhat arbitrarily chosen.

A few grams of special "K" calcium carbonate were suspended in water, and after long shaking the reaction was found to be P_H 9.23. This is a little lower alkalinity than the value P_H 9.5 obtained by Sharp and Hoagland (*10*). The reading P_H 10 denotes a higher alkalinity than that found in a normal soil.

The electrometric measurements then gave these data: The initial reaction of the soil suspension stated as P_H; the total number of cubic centemeters of calcium hydroxid (*N/25*) required to change the reaction to P_H 7, P_H 8.3, and P_H 10, respectively. The results of these measurements are recorded in Table II.

TABLE II.—*Initial reaction of the soil and the number of cubic centimeters of N/25 calcium hydroxid used to change the reaction to the figures given* [a]

GROUP 1. SOILS WHOSE INITIAL REACTION WAS MORE ALKALINE THAN IS INDICATED BY PH 8.3

Soil No.	County.	Soil type.	Initial P_H.	Cubic centimeters of $Ca(OH)_2$ required to titrate to—		
				P_H 7.	P_H 8.3.	P_H 10.
1169	Reno	Pratt loamy fine sand	8.61			0.7
1227	Greenwood	Crawford clay	8.56			5.4
1043	Russell	Benton loam	8.52			3.7
1221	Greenwood	Oswego silt loam	8.51			9.3
1297	Montgomery	do	8.46			2.5
1199	Jewell	Laurel very fine sandy loam	8.44			12.3
1119	Finney	Dune sand	8.44			4.4
1206	Jewell	Lincoln silty clay loam	8.40			4.0
1186	Reno	Kirkland clay loam	8.37			7.1

[a] Figures arranged according to increasing hydrogen-ion concentrations.

TABLE II.—*Initial reaction of the soil and the number of cubic centimeters of N/25 calcium hydroxid used to change the reaction to the figures given*—Continued

GROUP II, SOILS WHOSE REACTION WAS BETWEEN P_H 8.3 AND P_H 7

Soil No.	County.	Soil type.	Initial P_H.	Cubic centimeters of $Ca(OH)_2$ required to titrate to—		
				P_H 7.	P_H 8.3.	P_H 10.
1039	Russell	Summit silty clay loam	8.03		1.5	13.2
1219	Greenwood	Osage loam	7.81		3.0	10.1
1120	Finney	Sandy loam	7.50		2.0	6.8
1047	Brown	Silt loam (bottom)	7.49		.5	1.0
1132	Shawnee	Osage silty clay loam	7.45		2.7	8.7
1268	Montgomery	Osage clay (alfalfa land)	7.40		2.9	8.4
1267	do	Osage silt clay loam	7.36		3.0	19.1
1072	Harper	Brown loam	7.28		3.5	8.3
1160	Reno	Arkansas fine sand	7.26		1.4	3.5
1157	do	Arkansas clay loam	7.26		3.8	10.4
1070	do	Pratt loam	7.22		5.3	13.0
1131	Shawnee	Osage very fine sandy loam	7.19		2.0	4.6
1121	Finney	Finney clay	7.17		1.7	5.1
1162	Reno	Pratt fine sandy loam	7.05		6.9	16.1

GROUP III, SOILS WHOSE INITIAL REACTION WAS MORE ACID THAN IS INDICATED BY P_H 7

1287	Montgomery	Summit silty clay loam	6.77	2.5	5.3	11.4
1143	Shawnee	Boone fine sandy loam	6.76	.8	9.0	19.7
1191	Jewell	Colby silt loam	6.72	1.2	3.7	13.0
1190	do	Jewell silt loam	6.71	.8	3.6	7.9
1285	Montgomery	Crawford loam	6.65	1.0	4.2	12.5
1243	Cherokee	Cherokee silt loam	6.55	1.3	6.2	14.1
1230	do	Neosho silt loam	6.53	1.3	4.6	8.9
1058	Doniphan	Brown silt loam	6.53	1.3	5.1	10.2
1148	Reno	Dune sand	6.46	.6	2.3	4.2
1266	Montgomery	Bates very fine sandy loam	6.36	2.6	5.2	11.5
1271	do	Bates loam	6.29	2.4	6.5	20.2
1284	do	Crawford loam	6.25	.5	1.8	4.7
1141	Shawnee	Summit silty clay loam	6.15	6.7	17.5	31.4
1135	do	Crawford silty clay loam	6.01	8.4	20.0	35.2
1232	Cherokee	Bates silt loam	5.92	4.3	9.4	16.1
1136	Shawnee	Oswego silt loam	5.84	7.2	17.9	30.6
1256	Greenwood	Boone fine sandy loam	5.72	9.1	15.3	29.6
1279	Montgomery	Bates very fine sand	5.70	2.6	6.0	11.7
1265	do	Cherokee silt loam	5.56	6.7	10.3	23.2
1277	do	Bates shale loam	5.56	6.8	15.0	29.3
1280	do	Bates loam	5.54	3.1	6.0	13.5
1273	do	Bates very fine sandy loam	5.53	6.1	11.0	24.1
1257	Greenwood	Summit silty clay loam	5.49	9.2	22.0	35.3
1293	Montgomery	Bates stony loam	5.49	3.1	4.5	9.1
1275	do	Oswego silt loam	5.35	8.2	10.5	18.8
1233	Cherokee	Oswego silty clay loam	4.99	8.2	14.3	25.2
1244	do	Bates fine sandy loam	4.99	7.1	12.4	24.0
1239	do	Summit silt loam	4.68	9.8	19.3	31.0

CLASSIFICATION ON THE BASIS OF REACTION

When the data obtained, both in the calcium determinations and the electrometric measurements, were brought together it was found convenient to classify the soils into three groups. In group I were placed those soils whose initial reaction was more alkaline than is indicated by P_H 8.3. In group II were placed those soils whose initial reaction were less alkaline than is indicated by P_H 8.3 but more alkaline than is indicated by P_H 7. In group III were placed those soils whose initial reaction was more acid than is indicated by P_H 7. In arranging the soils within these groups the figures in Table I are given according to decreasing amounts of calcium soluble in N/1 hydrochloric acid. In Table II the soils are arranged according to decreasing alkalinity, or increasing acidity, as expressed by the P_H values.

CALCIUM CONTENT OF SOILS STUDIED

The soils of highest calcium content are found in group I. The four soils in group I which have as low calcium content as several of the soils in group II, or lower, are from the drier portion of the State. The soils in group III have an average lower calcium content than the soils in groups I and II. In general, the soils of a high calcium content have a more alkaline reaction than soils of low calcium content; yet because of the exceptions, the calcium content alone can not serve as the basis of classification as acid or alkaline. Most of the soils in group I are from the section of the State where acid soils are not usually found, whereas most of the soils from group III are from the section of the State where acid soils are more common. Sandstone-derived soils from the drier portions of the State may have a comparatively small amount of calcium and yet have an alkaline reaction.

In soils of high calcium content a larger percentage of the amount present is soluble in acid than in soils of low calcium content. As the percentage of total calcium decreases, it is relatively less soluble. This is true in comparing the groups and in comparing soils within groups. In group I the proportion of acid-soluble calcium is greater than in group II, and in group II it is greater than in group III.

The differences in the amounts of calcium in forms soluble in $N/1$ hydrochloric acid and in $N/5$ hydrochloric acid are small. For practical purposes they are of equal value.

The pronounced differences between the amounts of calcium soluble in hydrochloric acid and the total, especially in soils of low calcium content, raises the question of the relative importance of determining the total calcium in a soil or determining the amount soluble in cold dilute hydrochloric acid. These figures would indicate that the results obtained by the acid digestion are more valuable. In soils of low calcium content, it is present mostly in insoluble forms. While weathering gradually converts this calcium into forms that are soluble, the amount of available calcium obtained is insufficient for the needs of the soil. Such soils are deficient in "agricultural lime."

The figures for percentage of carbon dioxid show that all the soils in group I have some carbonates, that only 6 of the 14 soils in group II have carbonates in larger amounts than traces, and that only 3 of the soils in group III have carbonates in larger amounts than traces and in these the amounts are very small.

RESULTS OF ELECTROMETRIC MEASUREMENTS

The results on the electrometric measurements found in Table II are arranged according to decreasing alkalinity values or, which means the same thing, increasing acidity values. The figures expressing cubic centimeters of calcium hydroxid under the different P_H values in each case

mean the total calcium hydroxid used to bring the reaction to that point. In interpreting the results of these electrometric measurements the following factors must be considered: (1) Kind of soil with reference to the amount of sand, clay, and organic matter; (2) influence of climatic conditions; (3) amount of calcium present, particularly in the carbonate form. The amount of sand, silt, clay, or organic matter present in a soil may have a greater influence on the initial reaction than the amount of calcium present. Pratt loamy fine sand from Reno County has the lowest calcium content of the soils placed in group I, Table I, but it has the highest alkalinity as shown in Table II. Benton loam No. 1043 and Crawford clay No. 1227 are both high in calcium and both have a high initial alkaline reaction. The clay soils and the silty clay soils as a rule require more calcium hydroxid to change to a certain hydrogen-ion concentration than the sandy soils.

Soils placed in group III, Table II, are distinctly acid in reaction. As the initial acidity increases, the amounts of calcium hydroxid needed to change the reaction to neutral (indicated by P_H 7) also increases, but not uniformly. This is due to factors mentioned in the preceding paragraph. The influence of clay is shown by the figures in Table III.

Subsoils as a rule contain a larger amount of calcium than the surface soils, particularly calcium in the carbonate form. These same subsoils usually contain a larger amount of clay but a smaller amount of organic matter. The calcium was determined in a number of the subsoils corresponding to the surface soils mentioned in Tables I and II. The electrometric measurements were also made. The results are found in Table III. The figures for the surface soils are repeated from Tables I and II for the sake of comparison. The results in Table III are arranged within the groups with reference to the decreasing amounts of calcium in the surface soils. The results show that, with few exceptions, the subsoils have a higher calcium content than the surface soil and that in the majority of cases the subsoil requires a larger amount of calcium hydroxid to change it to the same reaction as the surface soil.

The soils in which the calcium content is less in the subsoil than in the surface soil are: 1297, Oswego silt loam; 1271, Bates loam; 1273, Bates very fine sandy loam; and 1277, Bates shale loam. In the first one of these soils the titration figure is larger for the subsoil than for the surface soil. This would be expected from the larger clay content and the smaller amount of calcium. The last two have sandy subsoils; and while no mechanical analyses were made, observations recorded at the time of taking the samples show that the subsoils have less clay than the surface soils. Both these soils were acid, and the subsoil is more acid than the surface soil. Yet the lesser amount of clay in the subsoil was of more influence in determining the amount of calcium hydroxid needed to bring to neutral reaction than the initial acidity.

TABLE III.—*Calcium content and electrometric measurements on subsoils in comparison with surface soils*

GROUP I, SOILS WHOSE INITIAL REACTION WAS MORE ALKALINE THAN IS INDICATED BY PH 8.3

Soil No.	County.	Soil type.	Calcium soluble in *N*/1 HCl, calculated to equivalent of $CaCO_3$ per acre 7 inches deep.	Initial PH.	Cubic centimeters of *N*/25 $Ca(OH)_2$ required to titrate to—		
					PH 7.	PH 8.3.	PH 10.
			Pounds.				
1043.1	Russell	Benton loam	200,500	8.52	.....	.....	3.7
1043.3			643,000	8.56	.....	.....	5.0
1227.1	Greenwood	Crawford clay	165,000	8.56	.....	.....	5.4
1227.3			206,000	8.75	.....	.....	8.0
1297.1	Montgomery	Oswego silt loam	57,000	8.46	.....	.....	2.5
1297.3			42,000	9.03	.....	.....	7.2
1199.1	Jewell	Laurel very fine sandy loam	30,000	8.44	.....	.....	5.3
1199.3			40,000	9.03	.....	.....	2.0
1206.1	....do	Lincoln silty clay loam	25,000	8.40	.....	.....	4.8
1206.3			45,500	8.40	.....	.....	7.6
1169.1	Finney	Pratt loamy fine sand	4,000	8.61	.....	.....	.7
1169.2			5,000	8.61	.....	.....	.5
		GROUP II, SOILS WHOSE INITIAL REACTION WAS BETWEEN PH 8.3 AND PH 7					
1085.1	Riley	Laurel silt loam	71,500	8.16	.....	0.7	7.9
1085.3			73,000	8.16	.....	.7	7.9
1047.1	Brown	Silt loam, bottom	33,000	7.49	.....	.5	1.0
1047.3			53,500	8.68	.....	.....	15.6
1039.1	Russell	Summit silty clay loam	31,000	8.03	.....	1.5	13.2
1039.3			78,500	8.28	.....	.....	17.7
1121.1	Finney	Finney clay	15,000	7.17	.....	1.7	5.1
1121.3			23,000	8.44	.....	.....	1.4
1070.1	Reno	Pratt loam	10,000	7.22	1.1	12.6	13.0
1070.3			14,000	6.67	.....	3.8	26.7
1157.1	....do	Arkansas clay loam	10,500	7.26	.....	3.8	10.4
1157.3			76,500	8.39	.....	.....	9.0
1120.1	Finney	Sandy loam	9,000	7.50	.....	2.0	.....
1120.3			105,500	8.92	.....	.....	.....
1072.1	Harper	Brown loam	9,000	7.28	.....	3.5	.....
1072.3			26,500	8.44	.....	.....	.....
1162.1	Reno	Pratt fine sandy loam	6,000	7.05	.....	6.9	.....
1162.3			8,500	6.95	.....	6.2	.....
		GROUP III, SOILS WHOSE INITIAL REACTION WAS MORE ACID THAN IS INDICATED BY PH 7					
1115.1	Finney	Richfield silt loam	16,000	6.57	0.8	10.4	.....
1115.3			177,000	8.34	.....	.....	.....
1287.1	Montgomery	Summit silty clay loam	14,000	6.77	2.5	5.3	.....
1287.3			17,000	6.13	1.7	3.8	.....
1058.1	Doniphan	Brown silt loam	13,000	6.53	1.3	5.1	.....
1058.3			14,000	6.32	4.0	15.3	.....
1190.1	Jewell	Jewell silt loam	12,000	6.71	.8	3.6	.....
1190.3			67,000	8.30	.....	.....	.....
1191.1	....do	Colby silt loam	11,500	6.72	1.2	3.7	.....
1191.3			38,500	8.44	.....	.....	.....
1135.1	Shawnee	Crawford silty clay loam	11,500	6.01	8.4	20.0	.....
1135.3			38,000	6.80	.....	.....	.....
1233.1	Cherokee	Oswego silty clay loam	8,500	4.99	8.2	14.3	.....
1233.3			11,500	4.42	10.3	21.6	.....
1257.1	Greenwood	Summit silty clay loam	8,500	5.49	9.2	22.0	.....
1257.3			23,000	7.90	.....	2.1	.....
1265.1	Montgomery	Cherokee silt loam	8,000	5.56	6.7	10.3	.....
1265.3			12,500	5.20	6.4	11.9	.....
1275.1	....do	Oswego silt loam	7,500	5.32	8.2	10.5	.....
1275.3			12,000	5.32	7.6	14.8	.....
1271.1	....do	Bates loam	10,500	6.29	2.4	6.5	.....
1271.2			7,000	5.46	3.1	6.5	.....
1273.1	....do	Bates very fine sandy loam	8,000	5.53	6.1	11.0	.....
1273.2			6,000	5.44	4.9	9.6	.....
1232.1	Cherokee	Bates silt loam	7,000	5.92	4.3	9.4	.....
1232.3			8,000	5.21	4.2	10.1	.....
1277.1	Montgomery	Bates shale loam	7,000	5.56	6.8	15.1	.....
1277.2			4,000	5.15	7.7	13.4	.....
1243.1	Cherokee	Cherokee silt loam	4,500	6.55	1.3	6.2	.....
1243.3			9,500	5.66	4.6	8.7	.....
1230.1	....do	Neosho silt loam	4,000	6.53	1.3	4.6	.....
1230.3			7,500	5.66	5.7	11.1	.....
1244.1	....do	Bates fine sandy loam	2,500	4.99	7.1	12.4	.....
1244.2			3,500	4.94	8.1	15.2	.....

Of the 34 soils represented in Table III, 13 required less calcium hydroxid for the titration of the subsoil than for the surface soil. Eight of these 13 soils have over three times as much calcium in the subsoil as in the surface soil. From the figures presented in Tables I and II, it is shown that when a soil has large amounts of calcium, especially in the carbonate form, the amount of calcium hydroxid used to bring to a certain reaction was less than when the calcium content was smaller. That is shown by comparisons of the groups. A large amount of calcium has a greater influence than a larger amount of clay.

The result on soil No. 1273 can be explained by the lesser clay content of the subsoil, as was pointed out in a preceding paragraph.

The following four soils have only slightly more calcium in the subsoil, and yet they require less calcium hydroxid for the subsoil than for the surface soil:

1199.3, Laurel very fine sandy loam.

1162.3, Pratt fine sandy loam.

1121.3, Finney clay.

1287.3, Summit silty clay loam.

The results on the first two may be explained by the lesser clay content of the subsoil. Finney clay 1121.3 is an abnormal soil. The sample was taken from the edge of a buffalo wallow. The probability is that the surface soil had more colloidal clay than the subsoil. Sample 1687 must be an exception; no explanation is apparant.

The foregoing presentation shows that most subsoils have a greater calcium content than the surface soil and also that the subsoils require a larger amount of calcium hydroxid to bring to the same reaction as the surface soil. This must be due to the absorptive power of the colloidal clay. It can not be due to a larger acid content or to a deficiency of basic elements. The larger content of calcium should neutralize the acidity, and since the calcium content is larger in the subsoil than in the surface soil in can not very well be said than the subsoil is more deficient in lime.

The initial reaction of a soil is not necessarily an indication of the amount of calcium hydroxid required to titrate to a given hydroxyl-ion concentration. In Table II the results are arranged according to the decreasing hydroxyl-ion concentration of the soil before titration. If these figures are studied in comparison with the figures in Table I it is found that the amounts used to titrate do not correspond to the initial reaction nor to the content of calcium except in the following general way. The soils placed in group I have a larger calcium content than the soils in group II, and those in group II have a larger calcium content than those in group III. The quantities of calcium hydroxid used in titration are greater for soils in group III than for soils in group II, and greater for those in group II than for those in group I. But for individual soils this comparison does not hold.

The total acidity in the soil was mentioned in a preceding paragraph as the total quantity of hydrogen ions which may be produced when the equilibrium is continually shifted by the introduction of hydroxyl ions. On such a basis it is possible to calculate the amount of lime required to satisfy this acidity as measured by the electrometric titration. It was also shown in a preceding paragraph that 1 cc. of N/25 calcium hydroxid used in titrating 10 gm. of soil is equivalent to 400 pounds of calcium carbonate per acre. Table IV has been prepared by using this factor and the titration figures from Table II. In the last column of Table IV are given the figures of the lime requirement of these soils as determined by the Hopkins method. It is at once seen that there is no close agreement in the figures obtained by the two methods. This does not necessarily argue for the greater practical value of the figures obtained by the electrometric method nor against the Hopkins method. Similar disagreements can be found if other well-known acidity methods are compared. The figures presented in Table III make it appear that some of the calcium hydroxid is taken up by colloidal clay. Just how much this amounts to is not known, nor the manner. This forms part of an investigation now going on at this laboratory.

Methods do not show any agreement as to the amount of calcium carbonate that should be added to an acid soil. Hopkins (7) states that 10 tons of limestone per acre on some soils is not too large a quantity. The figures in Table IV show that the amounts of lime required to change from a more acid reaction than denoted by P_H 7 to neutral, or P_H 7, is not in general larger than the figures obtained by the Hopkins method, though there is no agreement between individual samples. The amounts required to change from the initial reaction to that denoted by P_H 8.3 are not far from the amounts recommended for use on acid soils, and the amounts required to bring the reaction to P_H 10 are in all cases less than 10 tons per acre.

TABLE IV.—*Electrometric measurements in equivalents of $CaCO_3$ per acre in 0 to 7 inches of the surface soil in comparison with amount of $CaCO_3$ required by the Hopkins method*[a]

GROUP I, SOILS WHOSE ALKALINITY WAS ABOVE PH 8.3

Soil No.	County.	Soil type.	Initial PH.	Pounds per acre of $CaCO_3$ equivalent to titration of—			Pounds per acre of $CaCO_3$ required by the Hopkins method.
				PH 7.	PH 3.	PH 10.	
1169	Reno	Pratt loamy fine sand	8.61	.....	.....	280	.....
1227	Greenwood	Crawford clay	8.56	.....	.....	2,160	Alkali.
1043	Russell	Benton loam	8.52	.....	.....	1,480	.....
1297	Montgomery	Oswego silt loam	8.46	.....	.....	1,000	Alkali.
1199	Jewell	Laurel very fine sandy loam	8.44	.....	.....	4,920	.....
1119	Finney	Dune sand	8.44	.....	.....	1,760	.....
1206	Jewell	Lincoln silty clay loam	8.40	.....	.....	1,600	.....
1186	Reno	Kirkland clay	8.37	.....	.....	2,840	.....

[a] It is assumed that 1 cc. of *N/25* $Ca(OH)_2$ is equivalent to 400 pounds $CaCO_3$ per acre.

TABLE IV.—*Electrometric measurements in equivalents of $CaCO_3$ per acre in 0 to 7 inches of the surface soil in comparison with amount of $CaCO_3$ required by the Hopkins method*—Continued

SOILS WHOSE REACTION WAS BETWEEN PH 7 AND PH 8.3

Soil No.	County.	Soil type.	Initial PH.	Pounds per acre of $CaCO_3$ equivalent to titration of—			Pounds per acre of $CaCO_3$ required by the Hopkins method.
				PH 7.	PH 3.	PH 10.	
1085	Riley	Laurel silt loam	8.16	.....	280	3,160	.....
1039	Russell	Summit silty clay loam	8.03	.....	600	5,280	.....
1219	Greenwood	Osage loam	7.81	.....	1,200	4,040	.....
1120	Finney	Sandy loam	7.50	.....	800	2,720	.....
1047	Brown	Silty loam bottom	7.49	.....	200	400	.....
1132	Shawnee	Osage silty clay loam	7.45	.....	1,080	3,480	.....
1268	Montgomery	Osage clay (alfalfa land)	7.40	.....	1,160	3,360	340
1267	do	Osage silty clay loam	7.36	.....	1,200	7,640	340
1072	Harper	Brown loam	7.28	.....	1,400	3,320	.....
1160	Reno	Arkansas fine sand	7.26	.....	560	1,400	.....
1157	do	Arkansas clay loam	7.26	.....	1,520	4,160	.....
1070	do	Pratt loam	7.22	.....	2,120	5,200	.....
1131	Shawnee	Osage very fine sandy loam	7.19	.....	800	1,840	.....
1121	Finney	Finney clay	7.17	.....	680	2,040	.....
1162	Reno	Pratt fine sandy loam	7.05	.....	2,760	6,440	.....

SOILS WHOSE ALKALINITY WAS BELOW PH 7

1287	Montgomery	Summit silty clay loam	6.77	1,000	2,120	4,560	340
1143	Shawnee	Boone fine sandy loam	6.76	320	3,600	7,880	.....
1191	Jewell	Colby silt loam	6.72	480	1,480	5,200	.....
1190	do	Jewell silt loam	6.71	320	1,440	3,160	.....
1243	Cherokee	Cherokee silt loam	6.55	520	2,480	5,640	580
1230	do	Neosho silt loam	6.53	520	1,840	3,560	200
1058	Doniphan	Brown silt loam	6.53	520	2,040	4,080	.....
1148	Reno	Dune sand	6.46	240	920	1,680	.....
1266	Montgomery	Bates very fine sandy loam	6.36	1,040	2,080	4,600	680
1271	do	Bates loam	6.29	960	2,600	8,080	340
1284	do	Crawford loam	6.25	200	720	1,880	340
1141	Shawnee	Summit silty clay loam	6.15	2,680	7,000	12,560	.....
1135	do	Crawford silty clay loam	6.01	3,360	8,000	14,080	.....
1232	Cherokee	Bates silt loam	5.92	1,720	3,760	6,440	620
1136	Shawnee	Oswego silt loam	5.84	2,920	7,160	12,240	.....
1224	Greenwood	Summit stony loam	5.82	2,400	5,240	9,200	Alkali.
1256	do	Boone fine sandy loam	5.72	3,640	6,120	11,840	440
1279	Montgomery	Bates very fine sand	5.70	1,040	2,400	4,680	1,700
1265	do	Cherokee silt loam	5.56	2,680	4,120	9,280	1,700
1277	do	Bates shale loam	5.56	2,720	6,040	11,720	680
1280	do	Bates loam	5.54	1,240	2,400	5,400	2,380
1273	do	Bates very fine sandy loam	5.53	2,440	4,400	9,640	1,700
1257	Greenwood	Summit silty clay loam	5.49	3,680	8,800	14,120	480
1293	Montgomery	Bates stony loam	5.49	1,240	1,800	3,640	3,740
1275	do	Oswego silt loam	5.35	3,280	4,200	7,520	2,380
1233	Cherokee	Oswego silty clay loam	4.99	3,280	5,720	10,080	270
1244	do	Bates fine sandy loam	4.99	2,840	4,960	9,600	1,020
1239	do	Summit silt loam	4.68	3,920	7,720	12,400	490

SUMMARY

(1) A number of soils from different parts of Kansas were analyzed for total calcium, calcium in forms soluble in $N/5$ hydrochloric acid, and in $N/1$ hydrochloric acid. The amount of carbon dioxid in these soils was also determined.

(2) Ten-gm. samples of these soils were placed in 100 cc. neutral distilled water, and the initial reaction was determined by means of the

hydrogen electrode. *N/5* calcium hydroxid was added to change the reaction to a higher alkalinity. The points determined were the number of cubic centimeters of *N/25* calcium hydroxid needed to bring the reaction (if lower) to P_H 7, P_H 8.3, and P_H 10.

(3) In soils of a high calcium content, a larger percentage of the calcium is in forms soluble in these dilute hydrochloric acid solutions than in soils of a low calcium content.

(4) As a rule, soils of a high calcium content have a higher initial hydroxyl-ion concentration than soils of low calcium content.

(5) The amount of *N/25* calcium hydroxid required to change a soil from a lower to a higher hydroxyl-ion concentration depends more upon the amount of colloidal clay present than upon the calcium content.

(6) Subsoils, as a rule, have a higher calcium content than surface soils. It required more calcium hydroxid to change these subsoils from a lower to a higher hydroxyl-ion concentration than it did for the corresponding surface soils. This was true for most of the soils. The exceptions were due either to a very high calcium content in the subsoil as compared with the surface soil, or to a larger amount of sand in the subsoil, or to some unusual condition of the soil and subsoil.

(7) The amount of *N/25* calcium hydroxid required to change the acid soils to a reaction represented by P_H 7, calculated in equivalent pounds of calcium corbonate per acre, compares favorably with some other current methods of determining the lime requirements of the soil.

(8) In some soils the amount of calcium hydroxid, calculated in equivalents of pounds of calcium carbonate per acre, required to change to a concentration represented by P_H 8.3 is as great as the equivalent amount of acid-soluble calcium present in the soil, or greater.

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GREEN FEED VERSUS ANTISEPTICS AS A PREVENTIVE OF INTESTINAL DISORDERS OF GROWING CHICKS

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The problem of raising chicks in confinement has engaged the attention of many nutrition investigators for years. The difficulties encountered have been attributed to various causes, such as lack of vitamines in the feed, lack of exercise, and intestinal putrefaction. Whatever the causes may be it is recognized that they have proved a serious handicap in making use of the chick in nutrition work. The critical time in the life of a chick is between the ages of 8 and 12 weeks. During this period by far the greater mortality occurs when they are kept in confinement, and this is a most serious objection to their use in nutrition investigation. Drummond [1] has made some study of the growth of chicks in confinement and concludes that it is impossible to grow them successfully even when the feed is known to be suitable for growth. Osborne and Mendel [2] also report difficulty in raising chicks in confinement and have found the use of paper pulp to aid somewhat in lessening mortality. Hart and his associates [3] report difficulty in growing young chicks in confinement but have found no trouble in using birds weighing 3 or 4 pounds. The authors [4] have reported some success in raising chicks in confinement, but at that time it was thought the fair growth obtained was due to the green feed given in the ration. However, better results have since been secured without any green feed in the ration. The green feed was thougth to give the necessary succulence and add the vitamines needed for growth; but later experience does not indicate this to be true. The question now arises in the minds of the writers as to whether greens are necessary in the ration of a young growing chick. In three years' work with growing chicks in confinement there was no extra gain in weight or decreased mortality where sprouted oats were fed, over that of the control pens; in fact the chicks receiving greens were less vigorous than those in the other lots. It may be noted in this connection at Purdue University that in eight years of feeding 2-year-old steers in preparing them for the market there was no advantage gained, so far as the

[1] DRUMMOND, Jack Cecil. OBSERVATIONS UPON THE GROWTH OF YOUNG CHICKENS UNDER LABORATORY CONDITIONS. *In* Biochem. Jour., v. 10, no. 1, p. 77–88, 1 pl. 1916.

[2] OSBORNE, Thomas B., and MENDEL, Lafayette B. THE GROWTH OF CHICKENS IN CONFINEMENT. *In* Jour. Biol. Chem., v. 33, no. 3, p. 433–438, pl. 4–6. 1918.

[3] HART, E. B., HALPIN, J. G., and MCCOLLUM, E. V. THE BEHAVIOR OF CHICKENS FED RATIONS RESTRICTED TO THE CEREAL GRAINS. *In* Jour. Biol. Chem., v. 29, no. 1, p. 59. 1917.

[4] PHILIPS, A. G., CARR, R. H., and KENNARD, D. C. MEAT SCRAPS VERSUS SOYBEAN PROTEINS AS A SUPPLEMENT TO CORN FOR GROWING CHICKS. *In* Jour. Agr. Research, v. 18, no. 7, p. 391–398, pl. 50. 1920.

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average daily gain or selling price was concerned, by those steers receiving varying amounts of silage over those receiving only dry feed, except that the gains were made in the former case at a slightly reduced cost as compared with the latter, due largely to the fact that silage is cheaper than clover hay.

OBJECT OF THIS INVESTIGATION

Since sprouted oats seemed to be inefficient in preventing chick mortality, an attempt was made during the year 1919 to find some means of checking intestinal putrefaction, which postmortem examinations have shown to be the principal cause of mortality. Accordingly it was decided to try a series of different compounds which might be expected to have an antiseptic effect or might serve to prevent impaction by reason of their bulk.

THE EXPERIMENT

The stock used was 160 White Leghorn day-old chicks, which were divided into 10 lots of 16 chicks each. Every precaution was exercised to distribute the chicks so that they would be uniform in all lots. During the first four days the chicks in all lots were given water and granulated corn and had access to sand. Thereafter they were given their respective rations. At this time each bird was leg-banded and its weight was recorded. They were weighed individually at the end of each 14 days thereafter until the close of the experiment, at the end of 14 weeks. The weight of feed consumed by each lot was recorded each time the chicks were weighed.

The basal ration used was one which had proved most satisfactory during the past two years of feeding trials, including two different experiments—one with White Leghorns and the other with White Plymouth Rock chicks. All lots received the basal ration consisting of 50 parts cracked corn, 35 parts corn meal, 15 parts corn bran, 3 parts ash, 8.86 parts meat scrap, and 10.9 parts soybean meal (all parts by weight), and were provided with 1 inch of sand on the floor. In addition to this, some other factor was included in all lots, except in lot No. 1 which was the control pen. Lot No. 2 was provided with oat straw litter to note what effect the increased exercise or consumption of straw would have. Lot No. 3 was fed like No. 2, except that it received green feed in the form of tops of sprouted oats. The care of this lot represented the management usually given brooder chicks, since it provided a well-balanced ration and in addition supplied scratching litter and green feed. The exception to the usual brooder practice was that the birds were kept in confinement. Lots 3, 11, 13, and 14 are the lots reported in the tables as receiving green feed.

The idea has been advanced by some that the benefit of the scratching litter was derived not from the exercise it promoted but from the large quantities of the litter that were consumed by the birds, providing an

abundance of fiber which is considered so beneficial in the digestive tract. In view of this possibility, lots 4 and 5 were fed straw pulp. The only difference in the treatment of these two lots was that No. 4 received but one-half as much of the straw pulp as did No. 5. This pulp was prepared by taking strawboard (made of straw) and reducing it to a pulp with water. This pulp, after most of the water was expelled, was mixed with the dry mash. This was bulky, especially the mixture fed lot 5. The actual dry-weight consumption of paper was approximately 2½ and 5 per cent of the ration for lots 4 and 5, respectively. This pulp was palatable when mixed in the feed, and the chicks would eat it fairly well.

Lot 6 received the basal ration with hydrochloric acid added to the drinking water at the rate of 1 part 36 per cent hydrochloric acid (HCl) to 500 parts of drinking water. This is sometimes recommended as a substitute for buttermilk for use as a preventive or corrective of blackhead in turkeys and of bacillary white diarrhea and coccidosis in chickens.

Tobacco dust, a by-product of tobacco manufacturing and a valuable remedy against intestinal parasites, was given to lot No. 7 at the rate of 2 parts added to the basal ration. In like maner lot No. 8 received 2 parts of sulphur, and No. 9 received 6 parts of lactose. Lot No. 10 received the basal ration with copper sulphate added to the drinking water at the rate of 1 part copper sulphate crystals ($CuSO_4$) to 1,400 parts of water.

The mortality records and weights for the different lots are given in Table I.

TABLE I.—*Weight and mortality of chicks*

Lot No.	Ration.	Age, 8 weeks.		Age, 14 weeks.	
		Weight.	Mortality.	Weight.	Mortality.
		Gm.		*Gm.*	
1	Basal only	257	0	644	6
2	Basal+straw	252	3	475	8
3	Basal+straw and greens	257	3	524	8
4	Basal+2½ per cent straw pulp (No. 1)	247	3	630	6
5	Basal+5 per cent straw pulp (No. 2)	252	8	638	8
6	Basal+HCl	305	4	639	5
7	Basal+tobacco	223	4	535	6
8	Basal+sulphur	305	5	605	7
9	Basal+lactose	295	4	617	7
10	Basal+$CuSO_4$	306	2	653	2
11	Basal+greens [a]	243	1	384	11
12	Basal+greens [b]	186	3	360	11
13	Basal+no greens [c]	225	5	486	10
14	Basal+greens [d]	205	3	458	8

[a] Experiment No. I (1918), White Leghorns, fed same ration as Lot No. 3.
[b] Experiment No. II (1919), White Plymouth Rocks, fed same ration as lot No. 3.
[c] Experiment No. II (1919), White Plymouth Rocks, no greens; basal ration containing 10 parts of protein from meat scraps only, instead of meat scraps and soybean meal.
[d] Experiment No. II (1919), White Plymouth Rocks fed same as lot No. 13 with addition of greens.

FECES NITROGEN

A study of the nitrogen of the feces was made to note if any increased utilization or change in the nature of the nitrogen end products could be obtained because of the added compounds. The data from composite samples of the feces taken from the different lots are contained in Table II.

TABLE II.—*Amount and distribution of feces nitrogen*

Lot No.	Average protein consumed per chick in 14 days.	Percentage of total nitrogen.	Percentage of nitrogen soluble in *N*/10 HCl.[a]	Percentage of nitrogen insoluble in *N*/10 HCl.[b]	Percentage of soluble nitrogen in total nitrogen.
	Gm.				
1	58.5	2.23	0.94	1.29	42.1
2	56.78	Lost.			
3	68.11	2.20	.91	1.20	41.4
4	67.08	2.02	.94	1.08	46.5
5	73.63	1.77	.89	.88	50.3
6	67.23	1.83	1.15	.68	62.8
7	61.77	1.35	.85	.50	63.0
8	73.78	2.36	1.05	1.31	44.5
9	68.17	2.27	1.02	1.25	44.9
10	68.32	2.18	1.10	1.08	50.5

[a] Urea, ammonia, and amino acid nitrogen. [b] Uric acid and residual nitrogen.

DISCUSSION

Table I gives the results of the different rations outlined, including such factors as green feed, antiseptics, fiber, exercise, and their effect upon and mortality of the chicks. When the gain in weight and mortality of the different lots are considered, a few points stand out prominently and are suggestive as being worthy of further investigation.

The most important of these is the effectiveness of copper sulphate in preventing mortality, probably because of its well-known antiseptic properties. Since an antiseptic seems to be so effective, it adds additional evidence that one of the main causes of mortality of chicks grown in confinement is the intestinal putrefaction so often noticed in the autopsy of chicks. Sprouted oats is thought by some to be effective in lessening mortality, especially when fed for a short time only and when given as a supplement to a somewhat monotonous ration. It is possible that under the conditions of the experiment no benefit was obtained from its use with growing chicks when fed throughout the first 14 weeks of the growing period. Lots No. 11, 12, 13, and 14 noted in Table I include unpublished data obtained in previous experiments which are introduced here as further evidence of the ineffectiveness of greens in preventing chick mortality.

The sulphur received by lot 8 caused a continued looseness of bowels. This did not seem to have any ill effect and may have been of some advantage, since at 8 weeks of age this was one of the best lots. The retarding effect of tobacco was pronounced and resulted in stunting the growth during the first 8 weeks. There was a tendency for the chicks to

recover somewhat by the age of 14 weeks. The chicks in this lot always seemed more wild and nervous than those of any of the other lots.

The use of hydrochloric acid in the drinking water of lot 6 seemed to be of some benefit, inasmuch as the mortality was somewhat less than the average and the growth was consistent throughout the experiment.

Strawboard pulp was supplied to the ration in lots 4 and 5 for the purpose of adding bulk and thereby lessening the danger of impaction of the contents of the small intestine and caeca common when feeding a grain ration. It did not seem to aid in reducing mortality.

Lot 2 was given a litter of oat straw to encourage the chicks to exercise. This did not prove successful in promoting growth, since this lot made the smallest gain of all, nor did it tend to lessen mortality. Lot 1, which was the control lot, received only the basal ration. As shown in Table I, this ration has proved its efficiency in promoting growth and has also proved its inefficiency in checking mortality, especially during the time between the eighth and fourteenth weeks.

It will be noted from Table II that in lot 6 and also in lot 7, which received tobacco, the percentage of nitrogen in the feces was lower than in most of the other lots. Furthermore, it was found that the percentage of nitrogen excreted as uric acid was less, indicating a somewhat greater percentage of utilization of the nitrogen in the feed.

Lactose, which was added to the ration of lot 9 did not seem to aid in lessening mortality or in promoting growth. This may be due to the fact, as stated by Mendel and Mitchell,[1] that birds, unlike mammals, have no sugar-splitting enzyms in the small intestine; hence the sugar fed was not converted into lactic acid to any considerable extent and thus did not aid in checking intestinal putrefaction. This view is further substantiated in the production of the usual amount of uric acid in the feces, since otherwise nitrogen appearing as uric acid would probably have appeared as a soluble ammonium salt, as noted in lot 6 in Table II, where hydrochloric acid was used in the drinking water.

SUMMARY

(1) The tops of sprouted oats seem to be useless as a preventive of digestive disorders or as an aid to the growth of chicks in confinement.

(2) The analysis of the feces indicated that chicks given hydrochloric acid and tobacco powder produced less uric acid in their feces than did the other lots.

(3) Tobacco powder added to the ration of growing chicks prevents their normal growth and causes them to be wild and nervous.

(4) Hydrochloric acid, sulphur, and particularly copper sulphate offer interesting possibilities of success in raising chicks in confinement.

[1] MENDEL, Lafayette B., and MITCHELL, Philip H. CHEMICAL STUDIES ON GROWTH.—I. THE INVERTING ENZYMES OF THE ALIMENTARY TRACT, ESPECIALLY IN THE EMBRYO, *In* Amer. Jour. Physiol., v. 20, no. 1, p. 81–96. 1907. Bibliography, p. 94–96.

COMPARATIVE UTILIZATION OF THE MINERAL CONSTITUENTS IN THE COTYLEDONS OF BEAN SEEDLINGS GROWN IN SOIL AND IN DISTILLED WATER

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The experiments of Schröder on the distribution of organic and mineral constituents in seedlings of the kidney bean, *Phaseolus vulgaris*, published in 1868 [2] show that, in his fourth stage of germinating, when the second and third joints with the trifoliate leaves have formed, the cotyledons, which have become much reduced in size and more or less shriveled, still retain a considerable proportion of their mineral matter unused. Schröder's analyses show that these shriveled cotyledons retain about nine-tenths of their original calcium, whereas not more than one-fourth of their phosphorus and about two-fifths of their potassium, sodium, and magnesium remain. In regard to the calcium, however, Scröder points out that his determinations appear to be too high and that this result should be verified. In describing Schröder's experiments Pfeffer [3] remarks that—

> complete removal of all of the essential elements is never possible, for even in a starved plant, certain essential structural constituents can not be mobilized or consumed.

In 1915, the author of this paper published some results [4] showing that when the Kentucky Wonder garden bean was grown in distilled water, approximately 86 per cent of the calcium, 50 per cent of the phosphorus, and 40 per cent of the magnesium remained unused in the cotyledons as compared with the amounts found in the normal cotyledons. In this experiment the seedlings had been permitted to grow in distilled water until they became etiolated and died from lack of food. These figures approximate those given by Schröder.

The following experiment was undertaken with the view of comparing the degree of utilization of the total ash and the elements calcium, magnesium, and phosphorus, in the cotyledons of bean seedlings grown in distilled water and in garden soil.

In starting the experiment it seemed of primary importance to determine the distribution of the total ash and the elements calcium, magnesium, and phosphorus, which were to be studied, in the separate portions

[1] The author gratefully acknowledges Dr. A. M. Peter's careful criticism of this manuscript.

[2] Schröder, Julius. Untersuchung über die vertheilung des stickstoffs und der mineralbestandtheile bei keimung der schminkbohne. *In* Landw. Vers. Stat., Bd. 10, p. 493–510. 1868.

[3] Pfeffer, W. The physiology of plants . . . ed. 2, transl. and ed. by Alfred J. Ewart. v. 1, p. 584. Oxford, 1900.

[4] Buckner, G. Davis. Translocation of mineral constituents of seeds and tubers of certain plants during growth. *In* Jour. Agr. Research, v. 5, no. 11, p. 449–458. 1915.

of the bean under consideration. Since Schröder used the kidney bean, *Phaseolus vulgaris*, it was decided to use a kidney bean in this experiment, in order to obtain more comparable results. The Kentucky Wonder garden bean is a good example of this type, and, since it is well adapted to this climate, it was chosen.

About 3,000 perfect beans were selected and, after thorough washing, were allowed to soak in distilled water overnight, until the integuments were softened. From 1,000 of these beans the integuments were carefully removed and saved as a separate portion. The cotyledons were then carefully separated, and the embryos were dissected out. The 1,000 embryos and 200 of the cotyledons were separately analyzed, as were 400 integuments and 100 of the whole beans remaining. During these operations, care was taken that the separate portions did not become contaminated with dust or other foreign material. The materials were dried in an electric oven at 100° C. for 24 hours, after which they were weighed, ashed, and the phosphorus was determined by the method of the Association of the Official Agricultural Chemists,[1] while calcium and magnesium were determined according to the method of McCrudden.[2] All the analyses made during the progress of this experiment were similar in every respect. The results are stated in Table I, calculated for 1,000 beans and also as percentage of the moisture-free materials.

In determining the degree of utilization of the elements in question in the cotyledons of beans grown under normal conditions in garden soil, 500 carefully selected beans were planted in a box of garden soil in a room which received the proper amount of sunshine and ventilation. In this room, also, the seedlings in distilled water were grown. Since the room was used only for this purpose, the chance of contamination from dust during the growth of the seedlings was very small. When the bean seedlings had pushed the cotyledons well above the soil, the cotyledons were carefully washed with distilled water and a camel's-hair brush to remove any adhering soil. At all other times the watering was done from below, so that no water touched the cotyledons. As growth advanced, the cotyledons became greatly shriveled and turned brown and finally dropped off upon clean paper so placed as to keep them from falling on the soil. They were then analyzed and calculated according to the method described. The results will be found in Table I.

In that part of the experiment in which the seedlings were to be grown in distilled water, 1,000 beans from a new lot of the same variety (the first lot having been all used) were selected and sterilized by placing them in an atmosphere of formaldehyde gas for four hours, after which

[1] WILEY, H. W., et al. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 3. 1908.

[2] MCCRUDDEN, F. H. THE QUANTITATIVE SEPARATION OF CALCIUM AND MAGNESIUM IN THE PRESENCE OF PHOSPHATES AND SMALL AMOUNTS OF IRON DEVISED ESPECIALLY FOR THE ANALYSIS OF FOODS, URINE AND FECES. *In* Jour. Biol. Chem., v. 7, no. 2, p. 83–100. 1910.

they were washed with sterile, distilled water and germinated between blotting papers which had been treated with hydrochloric acid and washed free from chlorids with distilled water. The germinating dish was of porcelain and was sterilized by heating at 180° C. for two hours. The beans were allowed to germinate until the radicles were 1 cm. in length, when the integuments were removed and the radicles wrapped in sterile absorbent cotton which had previously been treated with hydrochloric acid and washed free from chlorids with distilled water. This cotton gave practically no ash when incinerated. After the radicles had been wrapped in the absorbent cotton, each bean thus prepared was placed in the mouth of a test tube which had been thoroughly coated inside with paraffin and was held there by applying a few drops of melted paraffin. The test tubes were thoroughly washed with distilled water before the distilled water in which the seedlings grew was placed in them. This water was replaced as rapidly as it was removed by evaporation and by transpiration. The bean seedlings were allowed to grow until they had etiolated and wilted. The seedlings thus formed were uniform in size and development, being about 7 inches in height, with a well-developed root system and having two perfectly formed leaves which were somewhat undersized. The etiolation of the leaves and cessation of growth was taken as a point of maturity at which the cotyledons were removed, in a brown and greatly shriveled condition. They were analyzed as already described, and the results are presented in Table I. Inasmuch as a new lot of Kentucky Wonder beans was used for this part of the experiment, 200 normal cotyledons from beans of this lot were analyzed and the results included in the table for comparison.

TABLE I.—*Analyses of whole beans and the several parts, calculated on the moisture-free material*

Material analyzed.	Dry matter.		Crude ash.		Phosphorus as P_2O_5.		Calcium as CaO.		Magnesium as MgO.	
	Gm.	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*
2,000 normal cotyledons	305.200	91.1	13.5300	4.43	4.9600	1.62	0.1989	0.06	0.6034	0.19
1,000 normal embryos	4.005	1.2	.1869	4.67	.0832	2.07	.0059	.15	.0129	.32
1,000 normal integuments	25.796	7.7	1.0467	4.05	.0360	.14	.5100	1.98	.1027	.40
Calculated for 1,000 beans	335.001	100.0	14.7636	4.40	5.0792	1.52	.7148	.21	.7190	.21
1,000 normal whole beans	329.013	100.0	17.8130	5.41	5.0464	1.53	.5914	.18	.8462	.26
2,000 exhausted cotyledons (grown in soil)	23.633	7.7	1.0730	4.54	.3500	1.48	.0372	.16	.0910	.38
2,000 exhausted cotyledons (grown in water) [a]	116.200	40.2	5.4850	4.72	1.9300	1.64	.1344	.12	.1574	.13
2,000 normal cotyledons [a]	278.000	100.0	11.9820	4.31	4.1000	1.47	.1557	.06	.3918	.14

[a] A different lot of Kentucky Wonder beans from those grown in soil.

In Table II will be seen the percentage distribution of phosphorus, calcium, and magnesium in the ash of the separate parts of the beans analyzed. Here we see that the percentages of phosphorus in the normal cotyledons and those exhausted under the given conditions are fairly constant, ranging from 32.62 in the exhausted cotyledons grown in soil to 36.66 in the normal whole cotyledons of the same lot. The percentage of phosphorus in the ash of the exhausted cotyledons of beans grown in distilled water very closely approximates that in the ash of the normal cotyledons of the same lot being 35.18 and 34.22, respectively.

TABLE II.—*Analyses of the ash of beans and of the several parts*

Material analyzed.	Phosphorus as P_2O_5.	Calcium as CaO.	Magnesium as MgO.
	Per cent.	*Per cent.*	*Per cent.*
Normal cotyledons	36. 66	1. 47	4. 46
Embryos	44. 50	3. 17	6. 88
Integuments	3. 44	48. 73	9. 81
Normal whole beans	28. 33	3. 32	4. 75
Exhausted cotyledons (grown in soil)	32. 62	3. 45	8. 48
Exhausted cotyledons (grown in water)[a]	35. 18	2. 45	2. 87
Normal cotyledons[a]	34. 22	1. 30	3. 27

[a] A different lot of Kentucky Wonder beans from those grown in soil.

In Table III will be seen the comparative amounts of dry matter, crude ash, and the elements phosphorus, calcium, and magnesium used by the seedlings grown in distilled water and those grown in garden soil. Here we see that 92.3 per cent of the dry matter, 92 per cent of the total ash, 92.8 per cent of the phosphorus, 81.4 per cent of the calcium, and 84.9 per cent of the magnesium of the cotyledons of beans grown in garden soil was translocated to other parts of the plant before the cotyledons ceased to function as a source of food supply. We see also that only 58.2 per cent of the dry matter, 54.2 per cent of the total ash, 42.9 per cent of the phosphorus, 14.1 per cent of the calcium, and 60 per cent of the magnesium in the cotyledons was utilized by the seedlings grown in distilled water cultures.

TABLE III.—*Comparison of percentages of material translocated from the cotyledons of beans grown in distilled water and in soil*

Material translocated.	In soil.	In distilled water.
Dry matter	92. 3	58. 2
Crude ash	92. 0	54. 2
Phosphorus	92. 8	52. 9
Calcium	81. 4	14. 1
Magnesium	84. 9	60. 0

It is readily observed that considerably more of each of these elements was utilized by the seedlings grown in garden soil than by those grown in distilled water. This would seem to indicate either that the distilled water is deleterious to the growth of seedlings grown in it or that something needed in the process of translocation was accessible when the beans were grown in soil but not when they were grown in distilled water.

Distilled water even of the highest purity has been considered toxic to seedlings grown in it, because of the difference between the osmotic pressure within the root cells and that of the distilled water surrounding them. The distilled water used in these experiments was obtained from a Barnstead automatic water still and contained traces of copper and calcium. In this case the toxic effect of the copper, if any could be attributed to it, was counteracted by the calcium, as there was no evidence of the characteristic poisonous effect of copper on the roots.

It is hoped that more light may be thrown on the subject of the utilization of the mineral constituents in the cotyledons by the young plant under varying conditions by experiments now in progress in this laboratory.

SUMMARY

When beans were grown in soil, a notably larger amount of reserve material was translocated from the cotyledons than when they were grown in distilled water.

In both cases, a smaller proportion of calcium was translocated than of phosphorus or magnesium.

SUNFLOWER SILAGE DIGESTION EXPERIMENT WITH CATTLE AND SHEEP[1]

By RAY E. NEIDIG, *Chemist*, ROBERT S. SNYDER, *Associate Chemist*, and C. W. HICKMAN, *Animal Husbandman, Idaho Agricultural Experiment Station*

The object of the experiment reported in this article was to determine the apparent digestibility[2] of silage made from sunflowers when fed to cattle and sheep. Sunflowers have gained a wide reputation as a silage crop in the Pacific Northwest, and much interest is being taken in their growth on lands where corn can not be successfully grown. Sunflowers are a hardier crop than corn, withstanding both drouth and frost to a much greater degree. Another point in favor of sunflowers is the fact that usually a greater tonnage can be secured in the semiarid regions. Many claims are made concerning the high value of sunflower silage for feeding purposes, but little is known at the present time as to its actual value other than numerous practical feeding tests which indicate that sunflowers are a very promising silage crop. Recently, however, the Montana Agricultural Experiment Station has reported on the digestible nutrients in sunflower silage made from a crop of sunflowers harvested when the plants were approximately 5 per cent in bloom. While a full report of the work has not been published, yet a summary of the digestible nutrients found in 100 pounds of silage, together with the same data on mature and immature corn, taken from Henry and Morrison's "Feeds and Feeding" is given in Bulletin 131 as follows:

	Total dry substance.	Crude protein.	Crude fiber and nitrogen-free extract.	Ether extract.	Nutritive ratio.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	
Digestible nutrients in 100 pounds of sunflower silage	21.4	1.24	10.13	0.37	9.8
Digestible nutrients in 100 pounds of mature corn[a]	26.3	1.1	15.00	.70	15.1
Digestible nutrients in 100 pounds silage from immature corn[a]	21.0	1.0	11.40	.40	12.3

[a] HENRY, W. A., and MORRISON, F. B. FEEDS AND FEEDING . . . ed. 17, X, 691 p. Madison, Wis., 1917.

From the digestible nutrients found in the sunflower silage and from the practical feeding experiments carried on by the Montana Agricultural Experiment Station, with dairy and beef cattle, ewes, and brood sows they conclude that sunflowers are a valuable silage crop.

[1] Published by the permission of Director E. J. Iddings, Idaho Agricultural Experiment Station, as a joint project of the Department of Agricultural Chemistry and Animal Husbandry.

[2] Throughout this article the coefficients of digestibility refer to the coefficients of apparent digestibility—that is, the difference in the weights of the nutrients of the silage fed and in the feces expressed in percentages of the total nutrients eaten.

During the past two years similar work has been carried out at the Idaho Agricultural Experiment Station, a part of which is reported in this article on the digestion experiments with cattle and sheep. The silage used was made from a crop of sunflowers harvested when about 50 per cent of the sunflowers were in bloom, but when only a few seeds were in the dough stage. The plan of the work and the data secured follow.

PLAN OF EXPERIMENT

Three registered Shorthorn cows, No. 5, 6, and 7, were used in the experiment. These were the only cows available at the time the experiment was conducted. Their ages varied, cow No. 5 being 3 years old, cow No. 6 being 10 years old, and cow No. 7 being 5 years old. These cows were kept in specially prepared stalls, which were arranged so that it was possible to obtain an exact record of all silage eaten, water consumed, all silage rejected, and all feces voided. No record was kept of the urine, either as to the amount voided or as to its chemical analysis.

Three yearling wethers, all pure-bred Shropshires, were placed in specially constructed pens which facilitated the securing of records on the amount of silage fed, silage eaten, water consumed, and feces voided.

The preliminary feeding period extended over a period of 10 days, during which time the animals were given an opportunity to accustom themselves to their surroundings, and also to ascertain the maximum amount of silage that they would consume daily. It was found that 50 pounds was the proper amount to feed the cows, while 2 pounds were sufficient for the daily sheep ration. The cows and sheep were fed one-half the full ration both morning and evening. When the animals appeared to be normal in every way a few days were allowed to elapse and then the final digestion period of seven days' duration was begun. During this period samples of the silage fed, silage rejected, and feces voided were collected daily and composited. Daily records of the amounts of silage fed, silage rejected, and feces voided were secured, together with the daily weights of the animals. Chemical analyses were made of all composite samples. The results are given on both the wet and dry basis in Table I.

Table II contains the amount of silage fed to cows and sheep, the water consumed, feces voided, silage rejected (called orts), and the daily weight of each individual cow and sheep. Table III contains the total weight of silage fed, the total nutrients contained in the silage eaten, and the feces voided. The amount of nutrients and the percentage digested are also given for each animal. In calculating the nutrients eaten, the total nutrients contained in the silage rejected were subtracted from the total nutrients contained in the silage fed. Hence the figures represent the actual amount of dry substance and nutrients eaten. The results are all expressed on the moisture-free basis.

TABLE I.—*Chemical composition of silage, orts, and feces*

	Moisture.	Dry substance.	Crude protein.		Crude fiber.		Ether extract.		Nitrogen-free extract.		Ash.	
			Wet basis.	Dry basis.	Wet basis.	Dry basis.	Wet basis.	Dry basis.	Wet basis.	Dry basis.	Wet basis.	Dry basis.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Silage fed	78. 79	21. 21	2. 03	9. 59	6. 30	29. 72	1. 23	5. 82	9. 56	45. 03	2. 09	9. 84
Orts rejected:												
Cow 5	69. 08	30. 92	1. 47	4. 77	15. 8	51. 1	. 43	1. 40	11. 41	36. 88	1. 81	5. 85
Cow 6	73. 12	26. 88	1. 37	5. 11	12. 67	47. 15	. 41	1. 54	10. 40	38. 66	2. 03	7. 54
Cow 7												
Sheep 2												
Sheep 3	72. 58	27. 42	1. 38	5. 04	13. 41	48. 9	. 43	1. 58	10. 21	37. 22	1. 99	7. 26
Sheep 8	69. 9	30. 1	1. 55	5. 14	13. 94	46. 3	. 57	1. 91	11. 89	39. 51	2. 15	7. 14
Feces voided:												
Cow 5	77. 64	22. 36	2. 20	9. 85	8. 73	39. 04	. 62	2. 93	7. 44	33. 10	3. 37	15. 08
Cow 6	78. 94	21. 06	2. 31	10. 99	7. 95	37. 75	. 67	3. 19	6. 95	32. 95	3. 18	15. 12
Cow 7	75. 46	24. 54	2. 58	10. 51	9. 21	37. 53	. 85	3. 46	8. 26	33. 66	3. 64	14. 84
Sheep 2	52. 09	47. 91	5. 12	10. 69	17. 88	37. 33	1. 49	3. 11	16. 00	33. 38	7. 42	15. 49
Sheep 3	56. 46	43. 54	4. 34	9. 96	16. 90	38. 81	1. 47	3. 37	14. 82	34. 05	6. 01	13. 81
Sheep 7	56. 27	43. 73	5. 19	11. 87	16. 87	38. 58	1. 36	3. 11	13. 69	31. 31	6. 62	15. 13

TABLE II.—*Feed and water consumed, feces voided, orts rejected, and daily weight of cows and sheep*

Date.	Silage fed to cows.	Water consumed by cows.			Feces voided by cows.			Orts rejected by cows.			Daily weight of cows.		
		No. 5.	No. 6.	No. 7.	No. 5.	No. 6.	No. 7.	No. 5.	No. 6.	No. 7.	No. 5.	No. 6.	No. 7.
	Kgm.	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Pounds.*	*Pounds.*	*Pounds.*
Apr. 5	22.68	.....	.....	.....	8.390	6.580	7.600	0.455	0.398	.....	1,050	1,170	1,170
6	22.68	.....	.....	.....	10.660	8.735	11.455	.195	.550	.....	1,050	1,170	1,170
7	22.68	.....	.....	.....	10.000	9.195	8.160	.306	.295	.....	1,040	1,188	1,173
8	22.68	.....	14.7	.....	10.445	10.325	11.000	.248	.488	.....	1,035	1,180	1,170
9	22.68	6.8	.....	.....	10.325	11.565	9.072	.263	.733	.....	1,040	1,175	1,173
10	22.68	.....	.....	.....	10.660	13.610	10.550	.244	.574	.....	1,033	1,195	1,165
11	22.68	.....	.....	.....	11.565	11.680	11.000	.156	.486	.....	1,045	1,168	1,155
Weight at end of period	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1,033	1,153	1,150

Date.	Silage fed to sheep.	Water consumed by sheep.			Feces voided by sheep.			Orts rejected by sheep.			Daily weight of sheep.		
		No. 2.	No. 3.	No. 8.	No. 2.	No. 3.	No. 8.	No. 2.	No. 3.	No. 8.	No. 2.	No. 3.	No. 8.
	Kgm.	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Pounds.*	*Pounds.*	*Pounds.*
Apr. 5	1.814	.....	.....	.....	0.383	0.462	0.355	.....	0.132	.....	123	131	112
6	1.814	.....	.....	.....	.332	.427	.201	.....	.....	.....	122	131	110
7	1.814	.....	.....	.....	.339	.413	.251	.....	.049	0.120	122	131	110
8	1.814	.....	.....	.....	.273	.376	.305	.....	.....	.....	122	130	110
9	1.814	0.342	0.342	.....	.357	.427	.261	.....	.045	.....	122	129	110
10	1.814	.....	.....	.....	.370	.427	.303	.....	.....	.....	121	130	111
11	1.814	.....	.....	.....	.333	.420	.284	.....	.....	.....	122	128	111
Weight at end of period	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	122	127	110

Table IV gives the apparent coefficients of digestibility for each animal, together with the average coefficients for the three cows and sheep, respectively.

Table V gives the pounds of digestible dry matter and pounds of digestible nutrients in 100 pounds of sunflower silage. The individual nutritive ratio for each animal is given, together with the average nutritive ratio for the cows and sheep.

TABLE III.—*Total weights of sunflower silage, feces, and water for the 7-day period*

[Results expressed in kilograms on moisture-free basis]

COW NO. 5

	Dry substance.	Crude protein.	Crude fiber.	Ether extract.	Nitrogen-free extract.	Ash.
Silage fed minus orts	33.096	3.203	9.714	1.952	14.848	3.279
Feces voided	16.109	1.586	6.293	.472	6.206	2.430
Amount digested	16.987	1.617	3.421	1.480	8.642	.849
Percentage digested	51.300	50.500	35.200	75.800	58.200	25.900

COW NO. 6

Silage fed minus orts	32.731	3.181	9.564	1.945	14.799	3.242
Feces voided	15.098	1.660	5.700	.482	7.511	2.282
Amount digested	17.633	1.521	3.864	1.463	7.288	.960
Percentage digested	53.900	47.800	40.400	75.200	49.200	29.600

COW NO. 7

Silage fed minus orts	33.673	3.229	10.008	1.960	15.163	3.313
Feces voided	16.893	1.776	6.342	.584	5.860	2.511
Amount digested	16.780	1.453	3.666	1.376	9.303	.802
Percentage digested	49.800	45.000	36.600	70.200	61.400	24.200

SHEEP NO. 2

Silage fed minus orts	2.694	0.258	0.801	0.157	1.213	0.265
Feces voided	1.144	.122	.427	.036	.382	.177
Amount digested	1.550	.136	.374	.121	.831	.088
Percentage digested	57.500	52.700	46.700	77.100	68.500	33.200

SHEEP NO. 3

Silage fed minus orts	2.634	0.255	0.772	0.156	1.190	0.261
Feces voided	1.286	.127	.499	.043	.439	.178
Amount digested	1.348	.128	.273	.113	.751	.083
Percentage digested	51.200	50.200	35.400	72.400	63.100	31.800

SHEEP NO. 8

Silage fed minus orts	2.664	0.256	0.787	0.156	1.202	0.263
Feces voided	.858	.102	.331	.027	.268	.130
Amount digested	1.806	.154	.456	.129	.934	.133
Percentage digested	67.800	60.200	57.900	82.700	77.700	50.600

TABLE IV.—*Coefficients of digestibility for cows and sheep*

[Expressed in percentages]

	Dry substance.	Crude protein.	Crude fiber.	Ether extract.	Nitrogen-free extract.	Ash.
Cow No.—						
5	51.3	50.5	35.2	75.8	58.2	25.9
6	53.9	47.8	40.4	75.2	49.2	29.6
7	49.8	45.0	36.6	70.2	61.4	24.2
Average for cows	51.7	47.8	37.4	73.7	56.3	26.6
Sheep No.—						
2	57.5	52.7	46.7	77.1	68.5	33.2
3	51.2	50.2	35.4	72.4	63.1	31.8
8	67.8	60.2	57.9	82.7	77.7	50.6
Average for sheep	58.8	54.4	46.7	77.4	69.8	38.5

TABLE V.—*Nutrients digested by cows and sheep in each 100 pounds sunflower silage*

[Estimated on wet basis]

	Dry substance.	Crude protein.	Crude fiber.	Ether extract.	Nitrogen-free extract.	Nutritive ratio.
Cow No.—	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	
5	10.9	1.03	2.22	0.93	5.56	9.6
6	11.45	.97	2.55	.93	4.71	9.6
7	10.56	.91	2.31	.86	5.87	11.1
Average for cows	10.97	.97	2.34	.91	5.38	10.1
Sheep No.—						
2	12.2	1.07	2.94	0.95	6.55	10.9
3	10.86	1.02	2.23	.89	6.03	10.1
8	14.4	1.22	3.65	1.02	7.43	10.9
Average for sheep	12.49	1.1	2.94	.95	6.68	10.6

INDIVIDUALITY OF COWS AND SHEEP AS TO THE AMOUNT OF SILAGE DIGESTED

An inspection of the tables shows that the three cows and three sheep all varied considerably in the amount of dry substances digested. In general, the same ratio of dry substance digested and nutrients absorbed existed. The sheep showed a much larger variation in total dry matter digested than was noted with the cows. The results of this one digestion period indicate that there exists an individuality among animals as to the thoroughness with which they digest their feed. This view is supported by the recent work of Grindley [1] and his associates on diges-

[1] GRINDLEY, H. S., CARMICHAEL, W. J., and NEWLIN, C. I. DIGESTION EXPERIMENTS WITH PIGS . . . Ill. Agr. Exp. Sta. Bul. 200, p. 55–94, 4 fig. 1917.

tion experiments with pigs, in which they found individual differences in pigs of the same age and species in the amount of feed digested which prevailed throughout 40 digestion periods.

It is readily seen that to secure an average digestion coefficient with any class of animals, a considerable number should be employed, which would mitigate the factor of errors introduced by individuality of the animals. If, however, a considerable number of animals are employed, the work becomes very voluminous and necessitates a large number of men to carry the experiment to completion. While these individual differences are not very great, it is thought that a sufficiently close digestive coefficient value can be obtained by using a smaller number of animals. In this work it is believed that the average coefficient obtained for the cows and sheep closely approximate the true digestive coefficient. A comparison of the analysis of the sunflower silage fed at this station and that fed at Montana, together with the digestible nutrients contained in each silage, follows.

TABLE VI.—*Comparison of sunflower silage fed at Idaho and Montana Agricultural Experiment Stations*

	Dry substance.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Ash.	Crude fiber and nitrogen-free extract.	Nutritive ratio.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	
Sunflower silage, Montana.....	21.4	2.1	6.8	10.4	0.5	1.6	.....	.....
Sunflower silage, Idaho.....	21.21	2.03	6.3	9.56	1.23	2.09	.....	.....
Digestible nutrients in 100 pounds silage, Montana.....	21.4	1.24	.....	.....	.37	.....	10.13	9.8
Digestible nutrients in 100 pounds silage, Idaho cows...	21.21	.97	.....	.....	.91	.....	7.72	10.1
Digestible nutrients in 100 pounds silage, Idaho sheep..	21.21	1.10	.....	.....	.95	.....	9.62	10.6

It is seen that a slight difference exists between the digestive nutrients found by Montana and those obtained by us, but the difference is small. No data are available as to the kind of animals used by Montana, hence no comments can be made. The nutritive ratio found by Montana and by Idaho is quite similar. Some of the difference is no doubt due to the different stages of maturity of the sunflowers. Montana silage was made from sunflowers cut when 5 per cent were in bloom, while Idaho silage represents a crop cut when 50 per cent were in bloom.

Additional studies are needed to determine the best time to cut sunflowers in order to secure the maximum food value.

When the digestion coefficients of sunflower silage obtained from cattle and sheep are compared with the coefficients of immature corn given in the early part of this paper, it is seen that for protein the cows utilizep practically the same amount from sunflower silage that they utilized from immature corn. With sheep, there is slightly more digestible protein

in immature corn silage. When sunflower silage is compared with mature corn, it is seen that the cows utilize slightly less protein from sunflowers than from corn silage, whereas sheep utilize similar amounts.

SUMMARY

(1) Analysis of sunflower silage fed at the Idaho Agricultural Experiment Station indicated that it compared very favorably with corn silage.

(2) The digestible nutrients contained in sunflowers compare favorably with the digestible nutrients in mature and immature corn.

(3) The nutritive ratio is somewhat narrower in sunflower silage than in mature or immature corn silage.

(4) Sheep utilized slightly more nutrients in sunflower silage than did cows under the conditions of this experiment.

(5) Where both corn and sunflowers can be grown, the selection of a silage crop should depend upon comparative tonnage per acre and cost of harvesting.

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